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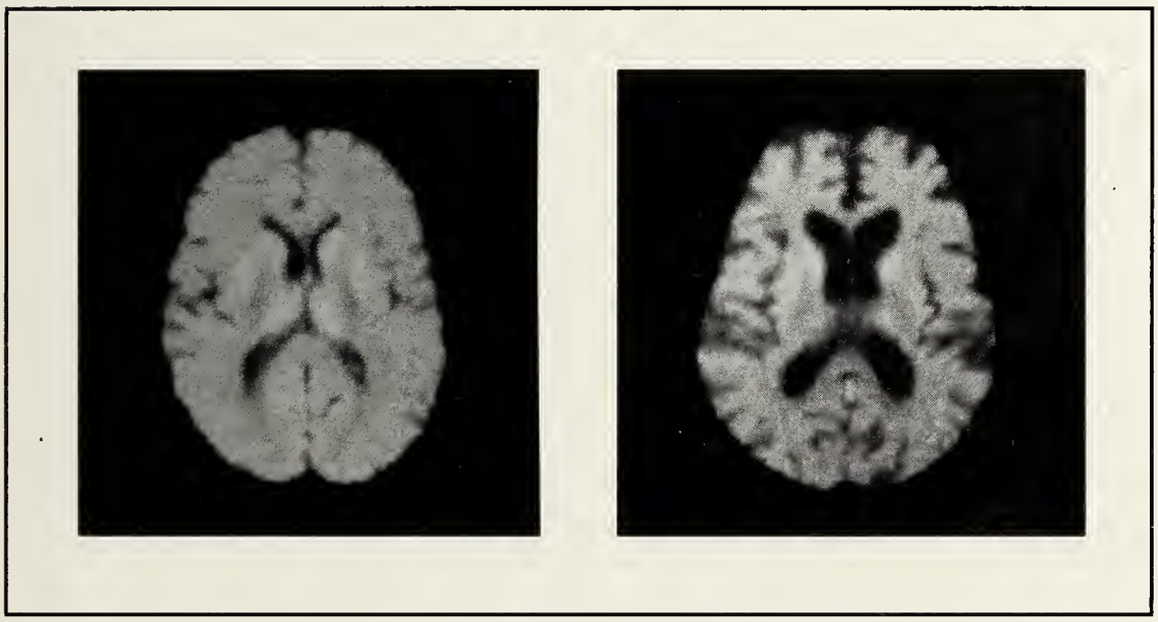

ALCOHOL-INDUCED BRAIN DAMAGE

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
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Research Monograph No. 22

ALCOHOL-INDUCED BRAIN DAMAGE

Sponsored by:
National Institute on
Alcohol Abuse and Alcoholism

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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Cover: *Axial magnetic resonance images from a healthy 57-year-old man (left) and a 57-year-old man with a history of heavy alcohol consumption (right). Images are courtesy of Dr. Adolf Pfefferbaum.*

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FOREWORD

Alcohol is used/abused by almost all cultures. Significant numbers of alcoholics exhibit some form of brain damage and/or cognitive dysfunction. The widespread use of alcohol and its capacity for producing negative consequences make continued study of the medical complications of alcoholism an important part of our national agenda. In this "Decade of the Brain," it is particularly fitting that a monograph that focuses specifically on the damaging effects of alcohol on the brain be compiled.

I commend the editors, Dr. Walter A. Hunt and Dr. Sara Jo Nixon, for recruiting outstanding scientists to participate in this project and for their efforts to integrate into a whole the wide range of topics this monograph explores. I also want to thank the contributors for their thorough discussions of the current issues in this field. Given the decades of research on alcohol-induced brain damage, consolidating the relevant literature into a meaningful volume is no small task.

I hope that interested readers will not only learn what is known but will also consider additional questions that may serve both to improve our understanding of brain/behavior relations during chronic alcohol abuse and to provide the basis for new treatment strategies for brain-damaged alcoholics.

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PREFACE

Alcohol is a neurotoxin. Although there is no evidence supporting the myth that each time one takes a drink a certain number of brain cells are lost, long-term alcohol abuse can lead to brain damage and cognitive and motor dysfunction. Because of the considerable progress over the last decade in defining the nature of these problems and, to a lesser extent, in elucidating the mechanisms involved, it is timely to review the status of this area of research.

The general goal of this monograph is to create a state-of-the-art treatise that provides a comprehensive, multidisciplinary review of alcohol-induced brain damage. The monograph is divided into several sections that include (1) an introduction that describes the prevalence and general characteristics of alcoholic brain damage; (2) a review of morphological, physiological, and cognitive abnormalities in humans; (3) a review of the anatomical, physiological, and biochemical changes associated with damage using animal models; and (4) possible molecular mechanisms of damage and potential therapeutic strategies to retard damage and/or enhance cognitive function.

Because the book is intended for a multidisciplinary audience, the chapters were written to minimize excessive detail yet be comprehensive, objective, and critical. Hopefully, the chapters will also focus the direction of future research by identifying gaps in our knowledge.

The preparation of a multiauthored book is often problematic because of different approaches and styles by individual contributors. This diversity can provide a variety of perspectives for the reader. As editors we appreciate this diversity and have attempted to limit our changes to those relevant to enhancing clarity.

In any book of this type, overlap is inevitable and is often desirable. Common subject matter discussed by different authors is valuable because it provides intellectual diversity, as well as the seeds for future research endeavors. Where topics are addressed briefly, references to other chapters with more complete discussions are included.

Heterogeneity within the field is reflected by the fact that two terms are used interchangeably, alcohol and ethanol, to refer to the toxin of interest. Alcohol is the term more frequently utilized by behavioral and social scientists, whereas ethanol is the term more frequently used by biological scientists. Given the multidisciplinary focus of this monograph, both terms are used.

Finally, we organized this monograph anticipating its use as a resource volume by a broad audience, including biological and behavioral scientists, clinicians, students, and others interested in the field. We hope that this monograph will be as stimulating to these readers as its preparation has been for us.

Without the support of others, this project would never have been completed. Thanks are extended to Lynn Adams and Roderic Ormond, to the National Institute

on Alcohol Abuse and Alcoholism for a Scientist Development award (K21 AA00134) to Dr. Nixon, and to our friends and family who provided support when work seemed endless. Finally, we thank the contributors who reviewed massive amounts of literature, distilling it into a cohesive whole to bring focus to what is frequently a confusing field.

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INTRODUCTION

THE EPIDEMIOLOGY OF ALCOHOL-INDUCED BRAIN DAMAGE

Mary C. Dufour, M.D., M.P.H.[1]

INTRODUCTION

Alcohol affects every organ and tissue of the body, either directly or indirectly (US DHHS 1990). Alcohol abuse is the fourth leading cause of death in the United States today, accounting for over 100,000 deaths annually (Shultz et al. 1990). The economic cost to society of alcohol abuse is staggering, totaling $86 billion in 1988 (Rice et al. 1990).

PREVALENCE OF ALCOHOL CONSUMPTION

In the United States, per capita alcohol consumption in 1989 was 2.43 gallons per person age 14 and older. By beverage type, this roughly translates into 311 12-ounce cans of beer, 67 5-ounce glasses of wine, and 166 1.5-ounce "shots" of 80-proof spirits for each man and woman age 14 and over (Williams et al. 1991). Per capita consumption is, however, a crude measure of actual patterns of alcohol consumption because it assumes that everyone in a given population drinks, which, of course, is not true. National drinking patterns in the United States, as derived from the 1988 National Health Interview Survey (NHIS), are shown in table 1 (Williams and DeBakey 1992). Malin et al. (1982) have estimated that of all drinkers, the 10 percent who drink most heavily consume 50 percent of the alcohol.

Analysis of the 1988 NHIS has also revealed, using the criteria of the *Diagnostic and Statistical Manual of Mental Disorders*, 3rd edition, revised (DSM-III-R) (American Psychiatric Association 1987), that 15.3 million Americans (8.6 percent of the general population age 18 and older) are either alcohol abusers or alcohol dependent (Grant et al. 1991). According to the National Drug and Alcoholism Treatment Unit Survey (NIDA/NIAAA 1990), 1.8 million individuals received treatment in the Nation's alcoholism treatment system in 1989. Many of these individuals exhibited symptoms of brain damage or brain dysfunction.

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METHODS FOR ESTIMATING PREVALENCE OF ALCOHOL-INDUCED BRAIN DAMAGE

Epidemiology is the study of a disease, condition, or injury in a population (Lilienfeld and Lilienfeld 1980). The epidemiology of alcohol-induced brain damage serves as an introduction to the topic by laying out the magnitude of the problem and providing a frame of reference for the material in subsequent chapters.

The brain is a major target of the actions of alcohol, and heavy alcohol consumption has long been associated with brain damage (Parsons 1977; US DHHS 1990). Nevertheless, the notion that we can discuss the epidemiology of alcohol-induced brain damage assumes that the population can be neatly divided into cases and noncases.

As this chapter unfolds, it will become readily apparent that clear-cut distinctions are often difficult to make. This difficulty is due to several factors, including (1) the lack of a linear relationship between the dose of alcohol and brain damage (Freund 1985); (2) the presence of mechanisms in the brain for dealing with injury (Freund 1985); (3) the fact that alcoholics are at increased risk for brain damage from a variety of causes, including poor nutrition, liver disease, and head trauma; (4) the existence of cognitive impairments that may antedate the onset of abusive drinking or be of unrelated etiology (Tarter and Edwards 1985); and (5) the fact that memory deficits may not be evident in "normal" detoxified alcoholics because the tests are not sufficiently difficult (Ryan et al. 1980). All of these factors suggest that the epidemiology of "pure" alcohol-induced brain damage is complicated and challenging.

TABLE 1

Distribution of levels of alcohol consumption in the population age 18 and older, U.S. 1988

Level of alcohol consumption	Men	Women
Abstainer (<12 drinks/year)	32%	53%
Light drinker (≥12 drinks/year to <3 drinks/week)	30%	30%
Moderate drinker (3-13 drinks/week)	25%	14%
Heavy drinker (≥2 drinks/day)	13%	3%

Note: A standard drink is defined as a 12-ounce can or bottle of beer, a 5-ounce glass of wine, or a 1.5-ounce shot of 80-proof distilled spirits. From Williams and DeBakey (1992).

Hospital and death records are two large national databases frequently used for epidemiologic research. The *International Classification of Diseases, 9th edition,* (ICD-9) (World Health Organization 1978) is used to code death records. In the United States, a modification of the ICD-9, called the *International Classification of Diseases, 9th edition, Clinical Modification,* or ICD-9-CM (ICD-9-CM 1980), is used to code hospital discharge records. The previously mentioned DSM-III-R is a diagnostic coding system frequently used by psychiatrists and other mental health professionals to label mental health conditions, including alcohol abuse. For this chapter, the DSM-

III-R, the ICD-9, and the ICD-9-CM are virtually identical. Therefore, only the ICD-9-CM codes will be discussed.[2]

PREVALENCE OF ALCOHOL-RELATED BRAIN DAMAGE

Three broad categories in the ICD-9-CM codes are used to describe alcohol-related disorders. They are alcohol abuse, alcohol dependence, and alcoholic psychoses. Approximately 9 percent of alcohol-dependent individuals have clinically diagnosable organic brain syndrome (Eckardt and Martin 1986), and up to 75 percent of detoxified long-term alcohol-dependent individuals show some degree of cognitive impairment (Eckardt and Martin 1986; Arria et al. 1990), with the specific prevalence varying with the sample and the methodology of assessment. Organic brain syndrome is an umbrella term that includes such entities as dementia, delirium, and amnestic syndrome. Alcoholic organic brain syndrome is roughly equivalent to the ICD-9-CM code for alcoholic psychoses. The most severe alcoholic psychoses can be identified easily because they are characterized by profound cognitive deficits.

Far more difficult is the identification of the much broader group of alcoholics and alcohol abusers who show no obvious behavioral evidence of brain dysfunction. Research findings among this group have been extremely variable, reflecting not only the great difficulty in studying subtle brain changes but also the hazards associated with searching for these deficits in an extremely heterogeneous population (Ryan and Butters 1986).

According to Caces et al. (1991), analysis of the National Hospital Discharge Survey (NHDS) (NIAAA 1989) data revealed that of 28,452,000 discharges in 1989 from short-stay community general hospitals for individuals age 14 and older, 1.3 percent, or 379,000, had a first-listed diagnostic code indicating an alcohol-related diagnosis; and 3.6 percent, or 1,022,000 discharges, had an all-listed alcohol-related code. (It might be noted that the record abstraction form permits the recording of up to seven conditions. The primary reason for hospitalization is the first-listed cause. "All-listed" refers to any of the up to seven conditions listed on the form.)

Alcoholic psychoses accounted for 14.0 percent of first-listed alcohol-related diagnoses and 5.1 percent of all-listed alcohol-related diagnoses. Over the years 1979–89, rates of discharge for alcoholic psychoses have remained stable. In 1987 there were 80,000 discharges with a first-listed diagnosis of alcoholic psychosis: 65,000 for men and 15,000 for women, yielding rates of 4.1 per 10,000 population for men and 1.5 per 10,000 for women. There were 164,000 discharges with an all-listed diagnosis of alcoholic psychosis. Discharges with a first-listed diagnosis of alcoholic psychosis had a coexisting diagnosis of alcohol dependence 38.8 percent of the time (NIAAA 1989). Cases with first-listed alcohol dependence had a coex-

[2] *The ICD-10 (1992) codes were recently released but will not be used in data collection in the United States until 2000. Therefore, the ICD-9-CM codes have been employed in the current chapter.*

isting diagnosis of alcoholic psychosis 7.0 percent of the time (NIAAA 1989). This 7-percent figure correlates well with the figure of 9 percent cited above for the proportion of alcoholics with organic brain syndrome.

It may seem strange to discuss hospitalizations in terms of the aggregate category of alcoholic psychoses because the specific disease entities included under this category, such as delirium tremens, Wernicke-Korsakoff syndrome (WKS) or psychosis, and alcoholic dementia, all appear to be separate and distinct diseases. In the NHDS, discharges are rarely investigated beyond the aggregate category of alcoholic psychoses because the numbers of cases become so small as to be statistically unreliable. Nevertheless, examination of the distribution of these diagnoses in the general hospital population is informative. According to Caces (personal communication, 1992), there were no short-stay general hospital discharges for WKS and very few for alcoholic dementia in 1987. The vast majority of discharges were for symptoms of alcohol withdrawal. While these symptoms might be considered a type of temporary cognitive impairment, they do not represent the organic brain damage of interest in this chapter.

In addition to short-stay general hospital discharges, patients with alcohol-related diagnoses are significantly represented in psychiatric services. They comprise 15 percent of psychiatric inpatient visits, 4.9 percent of psychiatric outpatient visits, and 9.3 percent of psychiatric partial care services. They make up 6.0 percent of nursing home patients with mental disorders and 3.9 percent of all nursing home residents. Almost 72 percent of nursing home residents with mental disorders and 46.7 percent of all nursing home residents have organic brain syndrome, primarily Alzheimer's disease (Manderscheid and Sonnenschein 1990). It is quite likely, however, that many alcohol-related cases are mistakenly placed in this category by diagnosticians unfamiliar with the patient's history of alcohol abuse.

PREVALENCE OF ALCOHOLIC PSYCHOSES

Despite its apparent rarity among short-stay general hospital discharges, according to Eckardt and Martin (1986), alcoholic dementia is the second leading cause of adult dementia in the United States, accounting for 10 percent of the cases. In comparison, Alzheimer's disease is the leading cause, representing 40 to 60 percent of the cases; it afflicts 5 to 11 percent of the population over age 65 and up to 47 percent of those over age 85 (Yankner and Mesulam 1991).

Based on national death records, alcoholic psychosis is an extremely rare underlying cause of death. In 1988, deaths related to alcoholic psychosis accounted for only 370 out of just over 2,000,000 deaths.

Except for acute untreated Wernicke's encephalopathy, alcoholic psychoses are generally conditions that one dies "with" rather than "of." This probably accounts, at least in part, for the fact that this diagnosis is rarely recorded as an underlying cause of death. Jorm (1990) also reports

that dementing diseases are usually not a cause of death and that substantial underreporting of dementing conditions occurs on death certificates. Alcohol-related conditions are grossly underreported for a variety of reasons. These reasons include the lack of knowledge of a history of alcohol abuse and the fear of stigmatizing the decedent's family (Dufour 1984).

Further substantiation of the enormous magnitude of such underreporting is provided by numerous autopsy studies of the brains of diagnosed alcoholics. For example, in a study of 191 brains of alcoholic individuals, Goldstein and Shelley (1980) reported that only 22 percent had no brain damage. The remaining 78 percent of the cases revealed significant brain damage, with 37.5 percent of the cases having diffuse damage and 40.5 percent having lesions limited to one side of the brain.

PREVALENCE OF WKS

Long-term, very heavy alcohol consumption in combination with malnutrition, particularly thiamine deficiency, produces devastating and well-documented alcohol-related brain pathology—the classic alcoholic WKS or psychosis. Although WKS can result from other origins of thiamine deficiency, alcohol abuse remains the most common cause in the United States today (Reuler et al. 1985).

The major symptoms of the classic Wernicke stage of WKS are acute and include a global confusion state, abnormal gaze, and abnormal gait (ataxia) (Eckardt et al. 1981). Mortality is between 10 and 20 percent in the acute phase (Reuler et al. 1985). With prompt vitamin therapy, including massive doses of thiamine, the neurological symptoms will frequently show significant improvement. The abnormal gaze will improve within hours to days and the ataxia and confusion within days to weeks (Charness et al. 1989). Most patients are left with some degree of ataxia, slight residual eye problems, and a severely disabling memory disorder constituting the chronic phase of this disorder—Korsakoff's psychosis (Eckardt et al. 1981; Charness et al. 1989).

The most debilitating aspect of Korsakoff's psychosis is the memory disorder anterograde amnesia, in which the individual is unable to learn any new information encountered after the onset of the Wernicke stage of the illness (Eckardt et al. 1981). People encountered moments before are regarded as total strangers. A trip down the hall to the bathroom may result in them forgetting where their room or bed is. Besides the memory deficits, classic Korsakoff's patients are passive, apathetic, and lacking in affect and initiative. Not surprisingly, these patients often require institutionalization for the rest of their lives (Oscar-Berman 1990).

It used to be thought that Korsakoff's psychosis was a permanent, irreversible condition. Victor et al. (1971) have subsequently reported (in a series of 104 Korsakoff's patients followed for up to 10 years) that this is not always the case. Up to 46 percent of these individuals showed significant or complete recovery from their amnestic symptoms. Of these recovering patients, approximately half

ceased to be dependent on custodial or supervisory care. Among those who recovered, the most progress was made in the first year following diagnosis and treatment.

Recovery of cognitive function has also been observed with less severely impaired alcoholics. In general, those alcoholics who remain abstinent show a superior ability to recover lost cognitive function (Guthrie 1980; Parsons 1987). The issue of reversibility may seem unrelated to a discussion of epidemiology; however, it is relevant because if an individual can fluctuate from being a "case" to being a "noncase," it becomes even more difficult to quantify the size of the problem of alcohol-induced brain damage.

Rates of first hospital admission for WKS range from one admission per million adults per year in England; 6 to 11 per million in New York and Scotland; 16 per million in New Zealand, Western Australia, and Victoria, Australia; and 65 per million in Queensland, Australia (Victor et al. 1971; Torvik et al. 1982; Harper 1983; Charness et al. 1989; Bishai and Bozzetti 1986). Some researchers feel that the diagnosis of WKS is wrongly applied to nonspecific intellectual impairment and is thus overreported (Goldstein 1985). On the other hand, others feel that many cases categorized as dementia really represent WKS, making the diagnosis underreported (Bowden 1990). Nevertheless, it is sometimes stated that WKS is an extremely rare condition and that Wernicke's encephalopathy and Korsakoff's psychosis are completely separate and distinct entities.

Nowhere has the contribution of postmortem neuropathology been more important than in the study of WKS. Harper (1983) and Harper et al. (1986) examined an autopsy series of 131 brains showing the distinctive neuropathology of WKS. Of these cases, only 20 percent were diagnosed as such during life. In 97 cases with complete hospital records, only 16 percent manifested the classic triad of eye signs, ataxia, and mental signs once thought to be required for a diagnosis of Wernicke's encephalopathy. Most of the individuals were described as demented. Torvik et al. (1982) examined the brains of 70 cases of WKS diagnosed at autopsy. Of the 22 cases dying in the acute or subacute (Wernicke) phase, only one was diagnosed during life. Among the 20 cases of chronic (Korsakoff) lesions, only three were correctly diagnosed during life, most of the cases being diagnosed as having some degree of global dementia rather than the classic amnestic and personality signs of Korsakoff's. Harper (1983) also reported that an additional 25 percent of the cases of WKS will be missed at postmortem examination if the brain is only examined grossly and not microscopically.

If one uses the neuropathological diagnosis of WKS at autopsy as the standard by which to describe the prevalence of this condition in the general population, clearly the concept that this is an extremely rare condition needs revision. In Oslo, Norway, Torvik et al. (1982, 1987) diagnosed WKS in 0.8 percent of 8,735 individuals coming to autopsy and in 12.5 percent of diagnosed alcoholics. Additional autopsy prevalence rates

include 1.7 percent for New York, 2.2 percent for Cleveland, 2.1 percent for Sydney, Australia, and 2.8 percent for Western Australia (Charness et al. 1989; Harper et al. 1989).

Another general conclusion to be drawn from these autopsy studies is that Wernicke's encephalopathy and Korsakoff's psychosis are often not easily distinguishable clinically. Harper et al. (1989) hypothesize that most alcoholics who have been labeled as demented will turn out to have lesions of WKS at autopsy and propose that WKS be viewed as a progressive disorder in which each clinical or subclinical episode contributes to cumulative brain damage. In short, WKS is more common in the general population than previously thought, is relatively common in alcoholics, and in most of the cases, is an insidiously progressive disorder (Harper 1983). Lishman (1990) further emphasizes the role of subclinical disease. These findings imply that it may not be necessary to require such a rigorous distinction between the various entities classified under the category of alcoholic psychoses.

To date, the best documented prevalence rate in the general population for WKS is 6.5 per 100,000 population per year as ascertained from a hospital-based surveillance program in the Sydney, Australia, area. Prevalence rates of WKS from Australia cannot, however, be generalized to other populations such as the United States because the rates reported for Australia tend to be much higher. For example, 15 percent of mental hospital admissions in both Queensland and Southern Australia are reported to be for WKS (Harper et al. 1989). The reasons for this are not completely known. Several potential factors include the high frequency of binge drinking in Australia, the lack of fortification of basic foods such as flour and bread with thiamine, and the lack of thiamine in Australian beer, the beverage of choice (Harper et al. 1989). Because of the more common occurrence of WKS in Australia, health care professionals there are likely to have a higher index of suspicion and to be better at making the diagnosis. Thus, the true prevalence rates in the United States are likely to be lower than in Australia but higher than currently reported.

RELATIONSHIP OF ALCOHOL CONSUMPTION TO SEVERITY OF DAMAGE

In the discussion of the epidemiology of any alcohol-related condition, the question invariably arises about how much alcohol produces such a lesion. Extremely heavy alcohol consumption for a prolonged period is generally required to produce the most severe alcoholic organic brain disease. Jacobson (1990) compared a group of 25 men with alcoholic Korsakoff's psychosis to 25 male alcoholics without serious neuropsychological deficits and found that both age of onset and duration of heavy drinking correlated with Korsakoff's psychosis. Those with Korsakoff's syndrome began drinking 150 grams of alcohol a day (roughly 12 standard drinks a day) at age 25, compared with 34 grams for the non-Korsakoff's alcoholics. In addition, the Korsakoff's alcoholic men had been drink-

ing at that level for an average of 27 years, compared with 20 years for the non-Korsakoff's alcoholics.

It must be noted, however, that as with most alcohol-related chronic medical conditions, alcohol consumption is a necessary but not sufficient condition for the development of alcohol-induced brain damage. Individual susceptibility is highly variable and related to a number of factors: gender (women may be more susceptible to alcoholic brain damage—see Glenn, chapter 9); genetics (such as the hereditary defect in the enzyme transketolase, which appears to predispose certain individuals to the development of alcoholic WKS); environmental factors (such as head trauma or occupational toxic exposure); and sociodemographics (income to buy nutritious food).

The question then becomes: What level of alcohol consumption is safe to avoid alcohol-induced brain damage? Most studies of alcohol-induced brain damage are done in alcohol-dependent individuals. Does that mean that any drinking short of alcohol dependence is safe? This is another difficult question to answer. Studies of alcohol-induced brain damage are most commonly done in alcoholics for two basic reasons. First, such damage is most likely to occur in the heaviest drinkers, who are generally but not necessarily alcohol dependent. Second, individuals in treatment for either alcohol dependence or the neuropsychological sequelae represent a captive population convenient for study. Nevertheless, individual susceptibility to becoming alcohol dependent is very variable. Some people become dependent at much lower levels of consumption than others. It is widely recognized that alcohol-dependent individuals suffer from a broad spectrum of alcohol-induced cognitive impairments; however, as mentioned in the introductory portion of this chapter, most people who drink are not alcohol dependent. Studies of cognitive function in social drinkers have produced mixed results, but the general conclusion is that insufficient data are available to suggest significant cognitive impairment (Parsons 1986).

At what level, then, is an individual at risk for developing alcohol-induced brain damage? Until recently, few epidemiological studies have investigated this issue. Ryback (1971), in a now classic review, postulated the so-called continuum theory of alcohol-induced cognitive impairment. Parker and Noble (1977) and Parker et al. (1983) reported that performance by social drinkers on tests of abstracting and adaptive abilities was negatively associated with the amount of alcohol consumed per occasion. The pattern was strongest among heavy drinkers but was also observed among moderate and light drinkers. Bergman et al. (1983), however, reported no correlation between levels of alcohol consumption and neuropsychological and neuroradiological measures in a sample of the general population. In a subsequent comprehensive review of the literature, Parsons (1986) concluded that study results were quite variable and seldom reproducible and that more research was urgently needed on this difficult to investigate but important question. More recently Parker et al. (1991) have reported

decreased sober cognitive functioning in groups of both male and female social drinkers who consumed alcohol an average of three or more times a week. They also showed that this relationship between quantity of alcohol consumed per occasion and abstraction performance depended on the frequency of alcohol use and could not be accounted for by psychological stress.

SUMMARY AND CONCLUSIONS

The precise prevalence of alcohol-induced brain damage in the United States today is unknown. Attempts at estimating the prevalence vary with the case definition of alcohol-induced brain damage and the methods used to identify the cases. Since drinkers are exposed to the same genetic, environmental, and sociodemographic factors as nondrinkers, and since they are susceptible to the same spectrum of brain diseases as nondrinkers in the general population, it is very difficult to define a case of "pure" alcohol-induced brain damage (Ryan and Butters 1986). Nevertheless, on a population level, it is possible to make several general statements.

Slightly less than half of American women and roughly two-thirds of American men 18 years and older consume alcohol. Over 15 million Americans aged 18 and older, 8.6 percent of the general population, meet the DSM-III-R criteria for alcohol abuse and/or dependence. Of these, at least 1.8 million individuals are treated annually in the Nation's alcohol treatment units. Numerous studies report that 50 to 75 percent of sober, detoxified long-term alcohol-dependent individuals suffer from some degree of detectable cognitive impairment, whereas the remaining quarter show no recognizable cognitive deficits. Approximately 10 percent of alcoholics suffer from serious dementia and, according to autopsy studies, as many as 12.5 percent of diagnosed alcoholics will have brain lesions characteristic of WKS. The most precise general population prevalence estimates of WKS come from a hospital-based surveillance system in Sydney, Australia, which reports a rate of 65 per 100,000 population per year. Granted, it may be an overestimation of WKS to apply the same rates to the United States. However, since diagnosable WKS represents the most severe end of the spectrum of alcohol-induced brain damage, this estimate is merely the tip of the iceberg representing alcohol-induced cognitive impairment in the general population.

There is a growing appreciation of just how commonly brain damage affects alcoholics and just how diffuse this damage is (Lishman 1987). As one considers the number of alcoholics in treatment who display some degree of impairment, the number of alcoholics sick enough to seek treatment, the number of alcohol-dependent individuals detected in the general population, and finally the number of drinkers in the general population, the estimates of population-based rates of alcohol-induced brain damage mushroom exponentially. The rapid and exciting advances being made in this field as outlined in subsequent sections of this monograph will serve as the groundwork that will enable more accurate and precise quantification of the magnitude of the problem of alcohol-induced brain damage in the future.

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2

CLINICAL AND PATHOLOGIC OVERVIEW OF THE BRAIN DISORDERS IN ALCOHOLICS

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INTRODUCTION

The study of brain damage in alcoholism is an interdisciplinary endeavor involving neuropsychological studies, brain imaging, neuropathological examination, and animal experimentation. Each of these approaches provides an incomplete view of a complicated condition, but all are essential for a comprehensive understanding of the cognitive and neurological disorders of alcoholics.

Neuropsychological studies can document fluctuations in cognitive function over the lifetime of an alcoholic, but such studies must be carried out without complete knowledge of the pathologic lesions underlying these changes. Using neuropsychological techniques, serial studies can be performed on recently detoxified and abstinent alcoholics. Mild cognitive impairment can be demonstrated by neuropsychological testing in 50 to 70 percent of detoxified alcoholics (Martin et al. 1986), and several groups have reported that abstinence leads to an improvement in cognitive function, particularly in the first weeks after the cessation of drinking (Carlen et al. 1984; Muuronen et al. 1989).

Approximately 10 percent of alcoholics exhibit stable and severe cognitive dysfunction ranging from Korsakoff's amnestic syndrome, a selective anterograde and retrograde amnesia, to dementia. Both of these patterns of cognitive dysfunction have been documented in patients whose brains reveal only the lesions of nutritional deficiency. This neuropathologic observation suggests a complication in the neuropsychological study of alcohol's putative neurotoxicity. Specifically, populations of alcoholic patients with global dementia may include many individuals with undetected lesions of nutritional deficiency.

Neuroimaging clearly has the potential to improve the etiological classification of cognitive dysfunction in alcoholics (see Pfefferbaum and Rosenbloom, chapter 4). Magnetic resonance imaging (MRI) can visualize some of the specific

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macroscopic lesions of Wernicke's encephalopathy, central pontine myelinolysis, cerebellar degeneration, and Marchiafava-Bignami syndrome. MRI and MR spectroscopy can also visualize noninvasively serial changes in cerebral morphology and metabolism that follow detoxification in alcoholics and may correlate with improved cognition.

However, MRI will always have several important limitations in the study of alcoholic brain disorders. The histopathologic basis for fluctuating radiographic abnormalities can rarely be known with certainty. For example, MRI cannot identify the microscopic changes that characterize all Wernicke's cases or the subtle alterations in neuronal number, size, architecture, and connectivity that may result from ethanol neurotoxicity.

Neuropathologic examination is the only means of identifying the histopathologic features of brain damage in alcoholics (see Harper and Kril, chapter 3). However, autopsy material presents a lifetime record of insults to the brain of an alcoholic, including lesions unrelated to ethanol neurotoxicity. Clinical data concerning drinking history, cognitive performance, and neurologic function must usually be obtained by retrospective chart review. Failure to record certain physical signs in a particular chart could indicate that the signs were absent or were present but not appreciated. These deficiencies could be partially overcome by undertaking longitudinal clinical-pathological studies. However, such studies are extraordinarily difficult to do in alcoholics.

Cellular and animal models lack many of the deficiencies of human clinical and pathological studies. For example, experimental studies control for nutritional deficiencies and liver disease, and the dose and duration of exposure to ethanol are precisely determined. Behavioral abnormalities can be correlated with neuropathologic abnormalities. Moreover, in some instances, brain transplantation can be used to correct alcohol-induced memory deficits in animals (see Arendt, chapter 22). Uncovering cellular and molecular mechanisms of ethanol neurotoxicity in cultured cells provides a basis for discovering cellular markers of ethanol neurotoxicity in human brains. For example, evidence of N-methyl-D-aspartate (NMDA) neurotoxicity in cell cultures treated chronically with ethanol should prompt a search for excitotoxic cell death in brain regions endowed with high concentrations of NMDA receptors (see Crews and Chandler, chapter 17). The principal limitation of cellular and animal studies is their occasional lack of generalizability to different species, including humans.

Brain damage in alcoholics is clearly multifactorial. Ethanol and its oxidative metabolite acetaldehyde may directly damage the developing and mature nervous systems (Arendt et al. 1988*b*; King et al. 1988; Lieber 1988; McMullen et al. 1984; Streissguth et al. 1980). Nonoxidative metabolism of ethanol generates fatty acid ethyl esters, which accumulate differentially in various tissues and correlates with susceptibility to ethanol-induced damage (Bora and Lange 1993; Laposata and Lange 1986).

Ethanol is a rich source of nonnutritive calories, so that heavy drinking is often complicated by malnutrition and vitamin deficiency (Thomson et al. 1983; Victor et al. 1989). The diagnosis of Wernicke's encephalopathy, the neurologic disorder of thiamine deficiency, is frequently missed (Harper 1983; Torvik et al. 1982). Therefore, it is difficult to know whether cognitive dysfunction in an alcoholic arises from malnutrition or ethanol neurotoxicity. This problem is compounded by the lack of a specific pathological lesion suggesting ethanol neurotoxicity and the frequent coincidence of several neurological complications of alcoholism. These include hepatocerebral degeneration, malnutrition and Wernicke's encephalopathy, head trauma, central pontine myelinolysis, Marchiafava-Bignami syndrome, pellagra (a syndrome associated with niacin deficiency), and premorbid pathological conditions such as fetal alcohol syndrome (FAS) (Tarter and Edwards 1986; Victor et al. 1989).

Interactions among these conditions may also influence brain damage in alcoholism. Acetaldehyde may acetylate transketolase, reducing its activity (Pratt et al. 1990). Thiamine deficiency in turn accelerates ethanol metabolism and the production of acetaldehyde by increasing alcohol dehydrogenase activity (Impeduglia et al. 1987; Martin et al. 1985; Martin et al. 1993). Chronic ethanol administration potentiates the lesions of experimental thiamine deficiency (Zimitat et al. 1990), perhaps by increasing NMDA-receptor expression and excitotoxicity (Lovinger 1993). Furthermore, ethanol impairs the recovery of function from neural injury of diverse etiologies (Lind et al. 1988; Orona et al. 1988; Tjossem et al. 1987; West et al. 1982). Finally, genetic factors may influence the susceptibility of certain alcoholics to develop neurological complications. For example, genetically determined abnormalities in the thiamine-dependent enzyme transketolase may explain why only a subset of malnourished alcoholics develops the Wernicke-Korsakoff syndrome (Blass and Gibson 1977; Martin et al. 1993; Mukherjee et al. 1987).

In this chapter, I survey the pathologically distinct brain disorders of alcoholics, emphasizing the complementary role of clinical studies, neuroimaging, pathological examination, and animal experimentation. The remaining chapters provide a detailed description of alcohol-induced brain damage from the perspective of each of these disciplines.

WERNICKE'S ENCEPHALOPATHY

Wernicke's encephalopathy is a common neurological disorder that is caused by thiamine deficiency. Alcoholics account for most cases in the Western world (Victor et al. 1989). Thiamine deficiency in alcoholics results from a combination of inadequate dietary intake, reduced gastrointestinal absorption, decreased hepatic storage, and impaired utilization (Thomson et al. 1983). Only a subset of thiamine-deficient alcoholics develops Wernicke's encephalopathy, perhaps because they have inherited (Blass and Gibson 1977; Mukherjee et al. 1987) or

acquired (Jeyasingham et al. 1987) abnormalities of the thiamine-dependent enzyme transketolase that reduce its affinity for thiamine.

The detailed, regional pathology of Wernicke's encephalopathy has been outlined by Victor and colleagues (Victor et al. 1989). The characteristic lesions of Wernicke's encephalopathy occur symmetrically in structures surrounding the third ventricle, aqueduct, and fourth ventricle. The mammillary bodies are usually involved, and the dorsomedial thalamus, locus ceruleus, periaqueductal gray, ocular motor nuclei, and vestibular nuclei are also commonly affected. Lesions occur less frequently in the colliculi, fornices, septal region, hippocampus, and cerebral cortex, which may show patchy, diffuse neuronal loss and astrocytic proliferation. In about half the cases, sagittal sections through the cerebellum reveal selective loss of Purkinje cells at the tips of the folia of the anterior superior cerebellar vermis. These cerebellar changes are identical to those found in alcoholic cerebellar degeneration, where they can occur without other Wernicke lesions.

Acute Wernicke lesions can be identified by endothelial prominence, microglial proliferation, and occasional petechial hemorrhages. In chronic lesions, there are demyelination, gliosis, and loss of neuropil with relative preservation of neurons. Neuronal loss is most prominent in the relatively unmyelinated medial thalamus (Torvik 1985; Victor et al. 1989). Atrophy of the mammillary bodies is a highly specific finding in chronic Wernicke's encephalopathy and is present in up to 80 percent of cases (Victor et al. 1989).

It is unclear how thiamine deficiency causes brain lesions. Thiamine is a cofactor for transketolase, α-ketoglutarate dehydrogenase, pyruvate dehydrogenase, and branched-chain α-ketoacid dehydrogenase (Victor et al. 1989) and may also function in axonal conduction and synaptic transmission (Iwata 1982). Thiamine deficiency produces a diffuse decrease in cerebral glucose utilization (Hakim and Pappius 1983). Shortly before the development of structural brain lesions, vulnerable brain areas exhibit a burst of metabolic activity accompanied by local production of lactate (Hakim 1984; Hakim and Pappius 1983). This burst of glucose utilization could represent a shift from aerobic metabolism to rapid glycolysis due to reduced pyruvate dehydrogenase activity (Hakim and Pappius 1983). (+)-5-methyl-10,11-dihydro-5H-dibenzo(a,b)-cyclo-hept-5,10-imine hydrogen maleate (MK-801), a drug that blocks ion currents activated by NMDA-specific glutamic acid receptors (Choi 1988), reduces the neurologic signs and the severity and extent of lesions in experimental thiamine deficiency (Langlais and Mair 1990).

These data suggest that glutamate toxicity may cause structural lesions in Wernicke's encephalopathy (Butterworth 1989), as happens in a variety of other neurological disorders (Choi 1988). Indeed, extracellular concentrations of glutamate increase up to sevenfold in the medial thalamus and threefold in the hippocampus following seizures in thiamine-deficient rats (Langlais et al. 1992). The

observation that chronic ethanol treatment increases the density of NMDA receptors in specific brain regions (Grant et al. 1990; Gulya et al. 1991) suggests a possible mechanism whereby chronic ethanol ingestion could potentiate the excitotoxicity of glutamate in Wernicke's encephalopathy (Lovinger 1993).

The distinctive nature of the pathological findings in Wernicke's encephalopathy permits subclinical cases to be diagnosed postmortem. Autopsy studies have consistently revealed a higher (0.8 to 2.8 percent) incidence of Wernicke lesions in the general population than is predicted by clinical studies (0.04 to 0.13 percent) (Harper 1983; Victor et al. 1989). The observation that acute Wernicke's encephalopathy was correctly diagnosed before death in only 1 of 22 patients (Torvik et al. 1982) suggests that the classic clinical triad of encephalopathy, opthalmoplegia, and ataxia is either surprisingly rare (Harper 1983; Reuler et al. 1985; Torvik et al. 1982) or is not properly elicited and recognized (Harper et al. 1986; Victor et al. 1989).

In clinical studies, only one-third of patients with acute Wernicke's encephalopathy present with the classic clinical triad (Victor et al. 1989). Most patients are profoundly disoriented, indifferent, and inattentive, and some exhibit an agitated delirium related to ethanol withdrawal. Although fewer than 5 percent of patients exhibit a depressed level of consciousness, the course in untreated patients may progress through stupor and coma to death (Victor et al. 1989; Wallis et al. 1978). Ocular motor abnormalities including nystagmus, lateral rectus palsy, and conjugate gaze palsies occur in 96 percent, reflecting lesions of the oculomotor, abducens, and vestibular nuclei. Gait ataxia occurs in 87 percent and is likely due to a combination of polyneuropathy (noted in 82 percent of cases), cerebellar involvement, and vestibular paresis (Ghez 1969; Victor et al. 1989). In keeping with the restriction of cerebellar pathology to the anterior and superior vermis, ataxia of the arms and dysarthria or scanning speech are each observed in fewer than 20 percent of cases (Victor et al. 1989).

Autopsy-based studies also suggest a very high incidence (82 percent) of mental status abnormalities but much lower incidences of ataxia (23 percent), ocular motor abnormalities (29 percent), and polyneuropathy (11 percent) (Harper et al. 1986). The classic triad of clinical findings was identified retrospectively in only 17 percent of autopsy cases, and 19 percent showed none of the classic elements. Stupor or coma, hypotension, and hypothermia were predominant findings in unsuspected cases (Harper 1983; Lindboe and Loberg 1989; Torvik et al. 1982). The discrepancy between prospective clinical and retrospective autopsy-based descriptions of Wernicke's encephalopathy is likely due to excluding atypical presentations in clinical series and underestimating in autopsy series classic signs that were not properly elicited, recognized, or recorded.

Computed tomography (CT) occasionally reveals low-density diencephalic abnormalities in acute Wernicke's encephalopathy (McDowell and LeBlanc

1984; Mensing et al. 1984). In some acute cases, MRI demonstrates increased T2[2] signal surrounding the aqueduct and third ventricle, consistent with the localization of the pathologic lesions (Gallucci et al. 1990; Yokote et al. 1991). Atrophy of the mammillary bodies can be identified by MRI in approximately 80 percent of alcoholics with a history of classic Wernicke's encephalopathy (Charness and DeLaPaz 1987) (figure 1). It is not found in control subjects, Alzheimer's patients, or alcoholics without a history of Wernicke's encephalopathy (Charness and DeLaPaz 1987, 1988). Alcoholics with the Wernicke-Korsakoff syndrome have small mammillary bodies and normal hippocampal volumes, as measured by MRI, whereas the converse is true in nonalcoholic amnestic patients (Squire et al. 1990).

The ability to detect specific Wernicke lesions by MRI should prove valuable in studying the cognitive disorders of alcoholics (Charness and DeLaPaz 1987). The finding of small mammillary bodies in a mentally impaired patient indicates that nutritional deficiency has played a role in the cognitive disorder. This technique may also prove useful in the diagnosis of atypical chronic cases of Wernicke's encephalopathy (Charness and DeLaPaz 1988) and in elucidating prospectively the full clinical spectrum of the disorder.

MR studies comparing amnestic alcoholics, nonamnestic alcoholics, and controls identified increases in cerebrospinal fluid (CSF) volume, ventricular enlargement, and reductions in cortical and subcortical gray matter structures in both alcoholic groups (Jernigan et al. 1991*a*,*b*). The amnestic alcoholics showed particularly large decreases in the anterior diencephalon and mesial temporal and orbitofrontal regions (Jernigan et al. 1991*b*). Neuropsychological measures in the nonamnesic alcoholics did not correlate with gray matter volume in a variety of brain regions.

With prompt administration of thiamine, ocular signs improve within hours to days, and ataxia and confusion within days to weeks (Victor et al. 1989). This early response likely represents recovery from a biochemical rather than a structural lesion. A majority of patients are left with horizontal nystagmus, ataxia, and a potentially disabling memory disorder known as the Korsakoff amnestic syndrome. These sequelae may result from the accumulation of lesions during repeated subclinical episodes of thiamine deficiency (Bowden 1990; Harper 1983; Lishman 1981; Witt and Goldman-Rakic 1983), rapid development of irreversible lesions during a single acute episode (Witt and Goldman-Rakic 1983), or inadequate treatment of patients with low-affinity transketolase variants (Jeyasingham et al. 1987).

KORSAKOFF'S AMNESTIC SYNDROME

Approximately 80 percent of alcoholic patients recovering from classic Wernicke's encephalopathy exhibit the selective memory disturbance of Korsakoff's amnestic syndrome (Victor et

[2] *For discussion of T1 and T2, see Pfefferbaum and Rosenbloom, Chapter 4.*

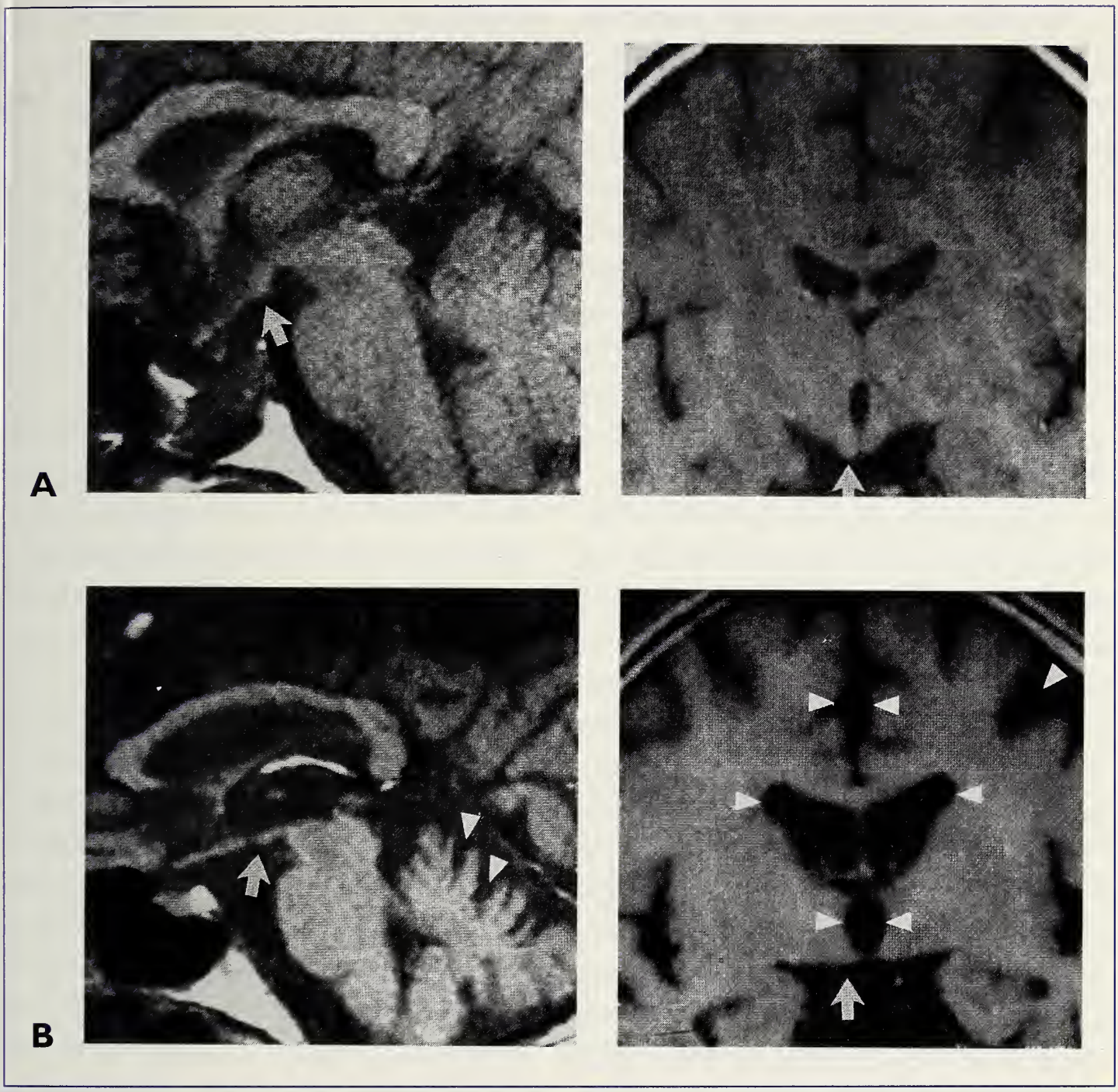

FIGURE 1

MRI of chronic Wernicke's encephalopathy. A, normal control; B, chronic Wernicke's encephalopathy. T1-weighted sagittal (left) and coronal (right) images in the plane of the mammillary bodies (arrows). The Wernicke patient shows shrinkage of the mammillary bodies (arrows) and anterior superior cerebellar vermis (arrow heads) and enlargement of the third ventricle, lateral ventricles, interhemispheric fissure, and cerebral sulci (arrow heads). Images were acquired using TR 600, TE 25 (an excellent description of MRI image acquisition, T1, and T2 can be found in Pfefferbaum et al. 1992). Reproduced with permission from Charness and DeLaPaz (1987).

al. 1989), which is characterized by marked deficits in anterograde and retrograde memory, apathy, an intact sensorium, and relative preservation of other intellectual abilities (Ekhardt and Martin 1986; Victor et al. 1989). Korsakoff's amnestic syndrome may also appear without an antecedent episode of Wernicke's encephalopathy (Harper 1983; Victor et al. 1989). Acute lesions may be superimposed on chronic lesions, suggesting that subclinical episodes of Wernicke's

encephalopathy may culminate in Korsakoff's amnestic syndrome (Bowden 1990; Harper 1979; Lishman 1981). The memory disorder correlates best with the presence of histopathologic lesions (Victor et al. 1989) and areas of decreased density on CT scan (Shimamura et al. 1988) in the dorsomedial thalamus. However, Wernicke lesions have been confined to the mammillary bodies in one reported case of Korsakoff's amnestic syndrome (Pittella and de Castro 1990).

Whereas Korsakoff's amnestic syndrome is most readily recognized as a relatively selective disorder of anterograde and retrograde memory, some alcoholics with Wernicke lesions exhibit a more global abnormality of higher cognitive function (Bowden 1990). A postmortem study of alcoholics, all of whom were psychiatrically evaluated during their illnesses, illustrates the difficulty in diagnosing these alcohol-related disorders (Torvik et al. 1982). In this study only 25 percent of 20 alcoholics with Wernicke's lesions had a circumscribed memory disorder. The rest had a global dementia. Of those patients diagnosed with dementia, 80 percent showed only the lesions of Wernicke's encephalopathy at autopsy, although subtle structural abnormalities were not sought (Torvik et al. 1982). The occurrence of global dementia in Wernicke's encephalopathy and the absence of non-Wernicke lesions in demented alcoholics has led some investigators to conclude that most cases of dementia in alcoholics are nutritional in origin (Lishman 1986; Torvik et al. 1982; Victor et al. 1989). Whether alcohol neurotoxicity plays a role in these cases can only be determined by correlating computerized brain morphometry with premorbid neuropsychiatric examination.

One of three patients in Wernicke's original report was a nonalcoholic (Victor et al. 1989). Wernicke's encephalopathy has since been described in nonalcoholic patients with malnutrition due to hyperemesis, starvation, gastric plication, renal dialysis, malignancy, and AIDS (Acker et al. 1982; Bjorneboe et al. 1988; Davtyan and Vinters 1987; Victor et al. 1989). Indeed, in one autopsy series, nonalcoholics accounted for 12 of 52 cases of Wernicke's encephalopathy (Lindboe and Loberg 1989).

Korsakoff's amnestic syndrome is an infrequent sequela of Wernicke's encephalopathy in nonalcoholics (Freund 1973). This observation has led to speculation that ethanol neurotoxicity is an important factor in the memory disorders of alcoholics (de Wardener and Lennox 1947). Although the neurotoxic effects of ethanol may worsen the cerebral disorder of thiamine deficiency, Korsakoff's amnestic syndrome can clearly occur without ethanol consumption. Accounts of thiamine deficiency in prisoners of war include descriptions of some individuals with enduring disorders of mental function following treatment with thiamine (Cruickshank 1950; de Wardener and Lennox 1947). More recent cases provide clearer descriptions of Korsakoff's amnestic syndrome following Wernicke's encephalopathy in nonalcoholics (Beatty et al. 1988; Becker et al. 1990; Engel et al. 1991; Parkin et al. 1991; Pittella and de

Castro 1990). Quantitative brain morphometry (see below) in these uncommon patients will indicate whether ventricular enlargement, shrinkage of cerebral white matter, selective neuronal loss, and simplification of dendritic arbors are specific lesions of ethanol neurotoxicity or previously unrecognized manifestations of thiamine deficiency.

CEREBELLAR DEGENERATION

Alcoholic patients may be afflicted by a chronic cerebellar syndrome related to the degeneration of Purkinje cells in the cerebellar cortex (Victor et al. 1956). Midline cerebellar structures—especially the anterior and superior vermis—are predominantly affected, a pattern that resembles the distribution of cerebellar pathology in Wernicke's encephalopathy (Victor et al. 1989). The cause of alcoholic cerebellar degeneration is not known with certainty, but its similarity to the cerebellar lesion in Wernicke's encephalopathy suggests that thiamine deficiency is likely an important factor (Mancall and McEntee 1965; Victor et al. 1956). It has also been proposed that alcoholic cerebellar degeneration, like central pontine myelinolysis, may be related to electrolyte abnormalities (Kleinschmidt-DeMasters and Norenberg 1981*a*). Evidence for a direct toxic effect of ethanol as the cause is poor. For example, cerebellar ataxia in alcoholics does not correlate with daily, annual, or lifetime consumption of ethanol (Estrin 1987). Furthermore, animal models of cerebellar degeneration induced by ethanol without nutritional deficiency show a different pattern of cerebellar pathology than in humans. In rats, the granule cells and molecular layer interneurons are more vulnerable than Purkinje cells (Tavares et al. 1987; also see Pentney, chapter 12).

Alcoholic cerebellar degeneration typically occurs only after 10 or more years of excessive ethanol use. It is usually a gradual, progressive disorder that develops over weeks to months but may also evolve over years or commence abruptly (Victor et al. 1956). Mild and apparently stable cases may become suddenly worse. As in Wernicke's encephalopathy, ataxia affects the gait most severely. Limb ataxia and dysarthria occur more often than in Wernicke's encephalopathy, whereas nystagmus is rare (Victor et al. 1956).

The diagnosis of alcoholic cerebellar ataxia is based on the clinical history and neurologic examination. CT or MRI scans may show cerebellar cortical atrophy (figure 1), but one-half of alcoholic patients with this finding are not ataxic on examination (Hillbom et al. 1986). Whether these represent subclinical cases in which symptoms will develop subsequently is unclear.

HEPATOCEREBRAL DEGENERATION

Hepatic encephalopathy develops in many alcoholics with liver disease and is characterized by altered sensorium, frontal release signs, asterixis, hyperreflexia, extensor plantar responses, and occasional seizures (see Tarter et al., chapter 21). Whereas some patients progress from stupor to coma and then death, others recover and suffer recurrent episodes. Brains of

patients with hepatic encephalopathy show enlargement and proliferation of protoplasmic astrocytes in the basal ganglia, thalamus, red nucleus, pons, and cerebellum, in the absence of neuronal loss or other glial changes (Victor et al. 1965).

Occasional patients do not recover fully after an episode of hepatic encephalopathy, but go on to develop a progressive syndrome of tremor, choreoathetosis, dysarthria, gait ataxia, and dementia. Hepatocerebral degeneration may progress in stepwise fashion, with incomplete recovery after each episode of hepatic encephalopathy, or slowly and inexorably, without a discrete episode of encephalopathy. Brain examination reveals astrocytic proliferation; laminar necrosis in the cortex; patchy loss of neurons throughout the cortex, basal ganglia, and cerebellum; and cavitation of the cortico-subcortical junction and superior pole of the putamen (Victor et al. 1989, 1965).

MARCHIAFAVA-BIGNAMI SYNDROME

Marchiafava-Bignami syndrome is a rare disorder characterized by demyelination or necrosis of the corpus callosum and adjacent subcortical white matter. This syndrome occurs predominantly in malnourished alcoholics (Brion 1976). Occasionally, there are associated lesions of Wernicke's encephalopathy or selective neuronal loss and gliosis in the third cortical layer. A few cases have been described in nonalcoholics (Kosaka et al. 1984; Leong 1979), demonstrating that ethanol alone is not responsible for the lesion. The course may be acute, subacute, or chronic and is marked by dementia, spasticity, dysarthria, and inability to walk. Patients may lapse into coma and die, survive for many years in a demented condition, or occasionally recover (Delangre et al. 1986). The disorder was formerly diagnosed only at autopsy, but lesions can now be imaged using CT or MRI (Kawamura et al. 1985). MR typically shows areas of increased T2 signal (figure 2) or cystic areas of decreased T1 signal within the corpus callosum (Kawamura et al. 1985).

CENTRAL PONTINE MYELINOLYSIS

Central pontine myelinolysis is a disorder of cerebral white matter that usually affects alcoholics. It also occurs in nonal-

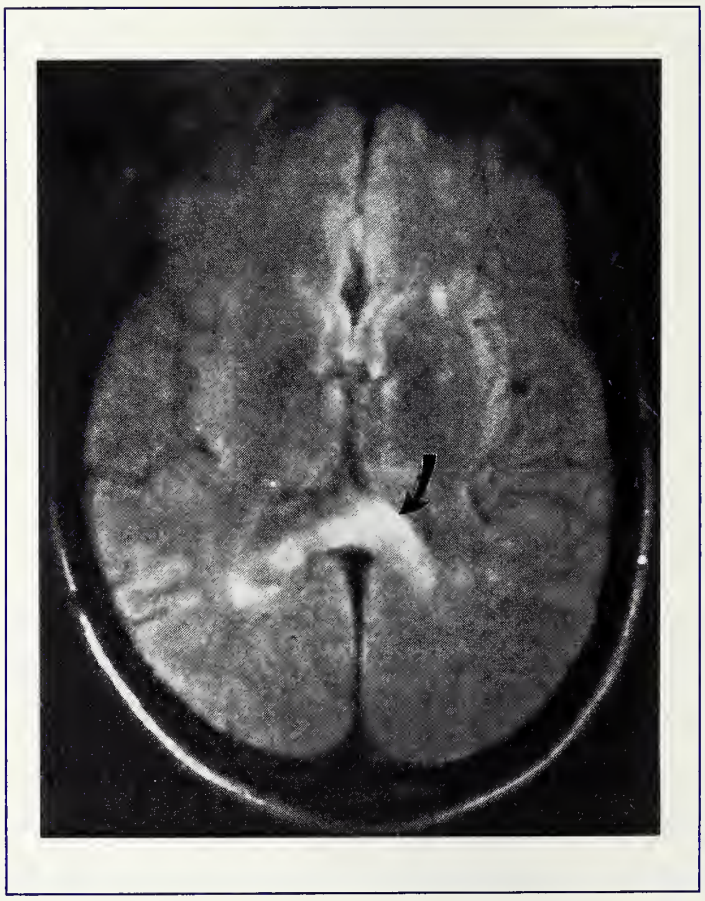

FIGURE 2

Marchiafava-Bignami syndrome. The splenium of the corpus callosum shows an area of increased signal (arrow). (TR 2000, TE 40). Reproduced with permission from Charness (1993).

coholics with liver disease, Wilson's disease, malnutrition, anorexia, burns, cancer, Addison's disease, and severe electrolyte disorders, such as thiazide-induced hyponatremia (Adams et al. 1959; Victor et al. 1989). Central pontine myelinolysis is frequently associated with a rapid correction of hyponatremia. However, most of the cases occur in alcoholics, suggesting that alcoholism may contribute to the genesis of central pontine myelinolysis in yet undefined ways.

The most common macroscopic lesion is a triangular region of pallor in the base of the pons. Approximately 10 percent of human cases also have symmetric extrapontine lesions, most frequently in the striatum, thalamus, cerebellum, and cerebral white matter (Wright et al. 1979). Microscopic examination reveals demyelinated axons with preserved cell bodies, except in the center of lesions, which may show cavitation. Myelinolytic lesions can be induced experimentally by rapid correction of chronic hyponatremia (Illowsky and Laureno 1987; Norenberg and Papendick 1984). In rats, the lesions are primarily extrapontine in location (Kleinschmidt-DeMasters and Norenberg 1981*b*), whereas in dogs, a mixture of pontine and extrapontine lesions is observed (Laureno 1983). The marked species differences in the distribution of lesions in central pontine myelinolysis illustrate one difficulty in generalizing pathologic findings from experimental animals to humans.

Symptoms and signs of central pontine myelinolysis may be absent (Adams et al. 1959) or obscured by associated conditions, such as ethanol withdrawal, Wernicke's encephalopathy, or hepatic encephalopathy. Treatment of these disorders may lead to an initial improvement in mental status, followed within days by confusion, lethargy, and coma due to central pontine myelinolysis. Involvement of the corticospinal tracts causes paraparesis or quadriparesis, and demyelination of the corticobulbar tracts leads to dysarthria, dysphagia, and inability to protrude the tongue. The tendon reflexes may be increased, decreased, or normal, and Babinski signs may be present. Disorders of conjugate eye movement occur occasionally and may reflect extension of the lesion in the pons or associated Wernicke's lesions. Disproportionate involvement of motor function may produce the "locked-in" syndrome, with only limited ability to move the limbs or face, despite a normal level of consciousness (Messert et al. 1979).

The lesions of central pontine myelinolysis can be visualized using CT scanning or MRI (figure 3). MRI is more sensitive than CT in imaging the pontine lesions (Miller et al. 1988). However, even MRI may be unremarkable early in the course of central pontine myelinolysis (Miller et al. 1988). The most common MR finding is an area of decreased T1 signal or increased T2 signal within the basis pontis. Other disorders, such as multiple sclerosis, multi-infarct dementia, and encephalitis, may show similar pontine MRI abnormalities but also cause significant periventricular abnormalities and a distinctive clinical picture (Miller et al. 1988). Serial CT or MRI studies suggest that the radiographic lesions resolve in

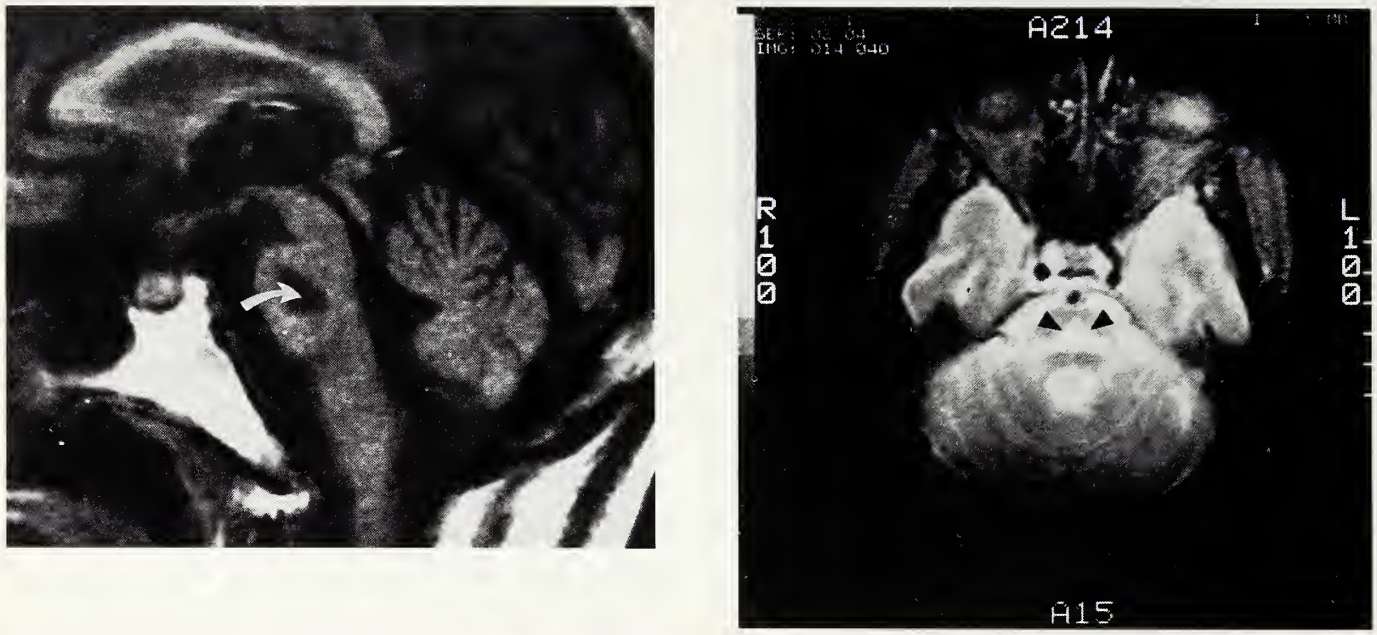

FIGURE 3

Central pontine myelinolysis. Arrows indicate a mid pontine abnormality which appears as an area of decreased T1 signal (sagittal image, left; TR 600, TE 25) and increased T2 signal (horizontal image, right; TR 2000, TE 70). Reproduced with permission from Charness (1993).

parallel with patient recovery (Charness and Diamond 1984; Miller et al. 1988). Therefore, the absence of lesions on MRI does not exclude the previous occurrence of central pontine myelinolysis.

ETHANOL NEUROTOXICITY IN THE DEVELOPING NERVOUS SYSTEM

Brain lesions in alcoholics may sometimes antedate birth. Alcoholism runs in families, and many alcoholics have been exposed to high concentrations of alcohol during critical stages of brain development. Any attempt to understand the pathogenesis of subtle brain lesions in alcoholics must consider the potential contribution of fetal alcohol exposure. FAS and its associated effects are not within the scope of this monograph and will not be discussed further. However, elucidation of MR abnormalities in FAS may help define the role of gestational exposure to alcohol in the cognitive disorders of alcoholics.

ETHANOL NEUROTOXICITY IN THE MATURE NERVOUS SYSTEM

Evidence that a direct neurotoxic effect of ethanol may contribute to chronic cognitive dysfunction in alcoholics has been obtained from imaging studies, neuropathological observations, and animal experiments. CT and MRI show enlargement of the cerebral ventricles and sulci in most alcoholics (Charness and Diamond 1984); however, when corrected for the effects of aging, the radiographic indices do not correlate consistently with either the duration of drinking or the severity of cognitive impairment (Victor et al. 1989). The ventricles and sulci become signifi-

cantly smaller within about 1month of abstinence (Carlen et al. 1984, 1978; Schroth et al. 1988; Zipursky et al. 1989), whereas brain water, estimated by MRI (Schroth et al. 1988) or chemical analysis (Harper et al. 1988*a*,*b*), does not change consistently. Based on these findings, it has been hypothesized that changes in brain parenchyma, but not brain water, may account for the reversible radiographic and cognitive abnormalities of alcoholics (Carlen et al. 1984; Schroth et al. 1988). In support of this hypothesis are reports that chronic ingestion of ethanol by well-nourished animals can reversibly reduce the complexity of dendritic arborization (McMullen et al. 1984) and alter the density of dendritic spines (King et al. 1988) in the hippocampus.

Pathologic studies of the brains of alcoholics have provided mixed evidence for ethanol-induced cerebral atrophy (Victor et al. 1989). Brain weight in alcoholics is reduced only slightly as compared with that in nonalcoholics (Harper et al. 1988*a*, 1985; Harper and Kril 1985; Torvik et al. 1982), and some studies have found no differences at all (de la Monte 1988; Victor et al. 1989). Brain volume—estimated by the volume of the pericerebral space, the CSF-filled region between the brain and skull—is reduced in alcoholics compared to controls. However, this indirect measure of cerebral atrophy is most abnormal in alcoholics with liver disease or Wernicke's encephalopathy (Harper and Kril 1985). Quantitative morphometry suggests that alcoholics, including those with liver disease and Wernicke's encephalopathy, lose a disproportionate amount of subcortical white matter as compared with cortical gray matter (Harper et al. 1985). This loss of cerebral white matter is also apparent when brains of nondemented alcoholics with liver disease are compared with those from patients with nonalcoholic liver disease. Hence, liver disease cannot be the sole cause of this selective loss of brain tissue. The loss of cerebral white matter is evident across a wide range of ages, is not accentuated in the frontal lobes (Harper et al. 1985), and is of sufficient magnitude (6 to 17 percent) to account for the associated ventricular enlargement (de la Monte 1988).

The identification of selectively affected neurotransmitter pathways could have important implications for the management of dementia in alcoholics. Cholinergic neurons in the nucleus basalis of the basal forebrain, which innervate much of the cerebral cortex and are preferentially depleted in dementia due to Alzheimer's disease, have also been reported lost in three patients with Korsakoff's syndrome (Arendt et al. 1983; also see Arendt, chapter 22). Levels of the acetylcholine-synthesizing enzyme choline acetyltransferase (CAT), which are reduced in the cerebral cortex in Alzheimer's disease, are also depleted in the brains of alcoholics, as reported in some (Antuono et al. 1980; Nordberg et al. 1980), but not all (Smith et al. 1988) studies. Whether putative cholinergic deficits in alcoholics are a consequence of thiamine deficiency or direct ethanol neurotoxicity is unclear.

Long-term administration of ethanol to rats causes memory deficits, reductions

in CAT levels and choline uptake, and a slight (17 percent) loss of neurons in the nucleus basalis (Arendt et al. 1988*a*,*b*). Transplantation of cholinergic neurons into the hippocampus and neocortex corrects both the cholinergic deficits and memory abnormalities, suggesting that, at least in rats, ethanol can directly damage cholinergic projection neurons (Arendt et al. 1988*a*, 1983). Evidence also exists for disruption of serotonergic and adrenergic pathways in amnestic alcoholics (Charness et al. 1989).

The recent use of computerized morphometry has revealed alterations in neuronal size, number, architecture, and synaptic complexity in alcoholics. Neuronal density in the superior frontal cortex was reduced by 22 percent in alcoholics compared to nonalcoholic controls (Harper et al. 1987). Neuronal loss was accompanied by selective glial proliferation in the superior frontal cortex and was more pronounced in a subgroup of alcoholics with cirrhosis or Wernicke's encephalopathy. By contrast, neuronal counts in motor, temporal, or cingulate cortex did not differ between the two groups (Harper et al. 1987; Kril and Harper 1989). A decrease in neuronal area was also observed in the superior frontal, cingulate, and motor cortices (Harper et al. 1987; Kril and Harper 1989). The complexity of basal dendritic arborization of layer III pyramidal cells in both superior frontal and motor cortices was significantly reduced in a group of 15 alcoholics compared to controls (Harper and Corbett 1990). Similarly, a significant reduction in dendritic arborization of Purkinje cells in the anterior superior vermis was found in four alcoholics, three of whom also had lesions of Wernicke's encephalopathy or pellagra (Ferrer et al. 1984). Finally, a group of five alcoholics without lesions of nutritional deficiency showed a decrease in the density of synaptic spines in layer V cortical pyramidal cells when compared to control patients (Ferrer et al. 1986).

These data demonstrate a selective neuronal loss, dendritic simplification, and reduction of synaptic complexity in different brain regions of alcoholics. It remains uncertain how these cellular lesions relate to the selective loss of white matter described above (however, see Lancaster, chapter 19). The etiology of these cellular lesions is also unclear, since patients often had coincident lesions of nutritional deficiency or cirrhosis. In fact, the cellular lesions were often most severe in the alcoholics with liver disease or Wernicke's lesions. There are three possible explanations for these findings: (1) liver disease and Wernicke's lesions are markers of worse alcoholism, greater ethanol intake, and more severe ethanol neurotoxicity; (2) liver disease and thiamine deficiency potentiate the neurotoxic actions of ethanol; or (3) liver disease and thiamine deficiency cause these lesions.

The fact that the neurological syndromes of liver disease and thiamine deficiency can develop slowly suggests that lesions in these disorders may also arise insidiously. Indeed, it is conceivable that some subtle, nonspecific lesions identified in alcoholic brains are early manifestations of malnutrition and liver disease

that precede more recognizable, specific lesions of Wernicke's disease and hepatocerebral degeneration. This hypothesis can be tested easily by using computerized morphometry to seek similar, subtle lesions in nonalcoholic patients with Wernicke's disease and hepatocerebral degeneration.

To date, all of the Wernicke patients studied by computerized morphometry have been alcoholics, some of whom have also had liver disease. Meanwhile, evidence that ethanol is neurotoxic must come from animal studies, where nutrition can be better controlled. Here, the data suggest that ethanol is neurotoxic in various rodent species, sometimes producing lesions similar to those observed in humans. Thus, with respect to hippocampal neurons, chronic ethanol treatment reduces (Walker et al. 1980) or does not change (McMullen et al. 1984) cell number, reversibly decreases (McMullen et al. 1984) or increases (Durand et al. 1989) dendritic complexity, and reversibly decreases or increases the density of synaptic spines (Durand et al. 1989), depending on the cell type (also see Walker et al., chapter 11).

THE EVALUATION AND TREATMENT OF COGNITIVELY IMPAIRED ALCOHOLICS

Alcoholics clearly are prone to many neurologic disorders that may impair cognitive function. The evaluation and treatment of alcoholics with abnormal mental status must consider all these conditions. Often, there are no historical clues to the duration and evolution of the condition, and not infrequently, patients deny that they have ever had a drinking problem.

A decreased level of consciousness should prompt a search for structural and metabolic abnormalities, particularly those more common in alcoholics (table 1). Our inability to reliably diagnose Wernicke's encephalopathy requires that all encephalopathic patients be treated with high doses of parenteral thiamine (100 mg intravenously for 5 days) (Charness et al. 1989). Intravenous glu-

TABLE 1

Frequent causes of altered mental status and depressed level of consciousness in alcoholics

Trauma
- Subdural hematoma
- Subarachnoid hemorrhage
- Intracerebral hemorrhage

Infection
- Sepsis
- Pneumonia with hypoxia
- Meningitis

Seizure
- Prolonged post-ictal state
- Nonconvulsive status epilepticus

Metabolic encephalopathy
- Hypoglycemia
- Hyponatremia
- Hypomagnesemia
- Hypothermia
- Wernicke's encephalopathy
- Hepatic encephalopathy
- Drug or toxin ingestion
- Ethanol or drug withdrawal
- Ethanol intoxication

Note: The list is compiled from the author's experience.

cose and naloxone should be administered to treat possible hypoglycemia and opiate intoxication. Fluid and electrolyte disorders must be identified and treated promptly, although symptomatic hyponatremia should be corrected slowly to avoid the development of central pontine myelinolysis (Charness et al. 1989). Toxicology studies of blood and urine should be sent. Spinal fluid should be examined to rule out meningitis. Stool should be examined for occult blood, and arterial blood gases should be drawn; the presence of occult blood and respiratory alkalosis is a clue to possible hepatic encephalopathy. In patients with focal neurological abnormalities, a CT head scan should be obtained to rule out structural lesions. Electroencephalography can diagnose nonconvulsive seizures and may provide clues to the nature of a metabolic encephalopathy.

In alcoholics with long-standing cognitive impairment and a normal level of consciousness, a different evaluation is warranted. Although the evaluation of dementia (Cummings and Benson 1992) is beyond the scope of this review, certain special considerations in alcoholics are worth noting. A history of previous episodes of Wernicke's encephalopathy or hepatic encephalopathy should be sought from family members and acquaintances, when available. Symptoms and signs of depression, overmedication, or covert drug ingestion should be elicited, since these are among the most common treatable causes of dementia. Blood work should include liver enzymes, vitamin B12, thyroid function tests, and syphilis serology. The sensitivity of neuropsychological assessment is reviewed elsewhere in this volume (see Parsons, chapter 8). The choice of a brain imaging study depends on cost, availability, and the importance of visualizing particular structures in an individual case. CT is cheaper and more widely available than MRI. However, MRI is superior to CT in detecting lesions of Wernicke's encephalopathy, central pontine myelinolysis, Marchiafava-Bignami syndrome, and vascular dementia. The mammillary bodies are not consistently visualized on routine MR studies. Therefore, thin sagittal and coronal cuts through the mammillary bodies should be requested (Charness and DeLaPaz 1987).

Abstention from drinking is the most important and most difficult goal in the treatment of long-standing cognitive impairment in alcoholics. Because thiamine deficiency may play a role in chronic cognitive impairment in alcoholics, as stated above, all such patients should be treated with parenteral thiamine in the short-term, and oral thiamine thereafter.

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BRAIN AND COGNITIVE CHANGES IN ALCOHOLIC SUBJECTS

3

NEUROPATHOLOGICAL CHANGES IN ALCOHOLICS

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INTRODUCTION

A range of neuropathological changes have been observed in the brains of chronic alcoholic subjects. Many of these subjects develop other medical complications because of their excessive alcohol intake, and these can secondarily affect the nervous system. The most notable examples are disorders associated with thiamine (vitamin B_1) deficiency and cirrhosis of the liver. Thus, although we can characterize pathological changes in the different anatomical regions of the nervous system in alcoholic subjects, it is difficult to dissect out the exact pathogenetic mechanisms causing the damage.

Many studies discussed in this chapter were carried out in Australia, a country said to have the highest per capita alcohol intake in the English-speaking world (Wodak 1986). To identify the major pathogenetic factors that are thought to cause alcohol-related brain damage, such as alcohol per se or its metabolites, associated vitamin B_1 deficiency, and liver failure due to cirrhosis, cases for study were categorized as outlined below. All cases were extensively screened to eliminate neurological abnormalities such as strokes, Alzheimer's disease, and severe head injuries. In most of the studies, the mean age of the alcoholic groups was in their fifties, and their drinking histories were more than 20 years.

Groups studied:

Control subjects, who consumed less than 20 grams of alcohol per day.

Moderate drinkers, who consumed between 30 and 80 grams per day.

Uncomplicated alcoholic subjects, who consumed greater than 80 grams per day but did not have pathological evidence of either thiamine deficiency (Wernicke-Korsakoff syndrome; WKS) or cirrhosis of the liver.

Alcoholics with WKS (pathologically confirmed).

Alcoholics with cirrhosis of the liver. (pathologically confirmed).

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These definitions may conflict with many other authors' ideas of, for example, "moderate drinking." Parker and Noble (1977) defined "moderate" as a much lower level of alcohol intake. Nevertheless, the moderate and uncomplicated alcoholic groups, as defined above, are particularly important subgroups. In a study of 515 patients seen in a tertiary referral alcoholic brain damage center in Australia, the proportion of professional and skilled individuals was the same as in the general population. Many of these would be defined as moderate drinkers or uncomplicated alcoholics and do not fit the archetypal "alcoholic" subject. Such a subject is often portrayed as demented, having severe sensory loss, and having difficulty walking because of cerebellar degeneration and peripheral neuropathy (Tuck and Jackson 1991). Recent clinical and neuropsychological studies have shown more subtle forms of alcohol-related brain damage, with impaired skills related to planning, organizing, abstracting, and problem solving (Walsh 1985; Waugh et al. 1989; Tuck and Jackson 1991). The identification of the pathological substrate causing this pattern of brain damage is obviously important, as there is good evidence to suggest that much of this damage is reversible if subjects abstain from excessive alcohol intake (Carlen et al. 1978; Jacobson 1986).

BRAIN WEIGHT AND VOLUME

One of the most notable abnormalities in the brains of alcoholics is the presence of brain shrinkage. This was first documented using pneumoencephalography (Brewer and Perrett 1971) and later confirmed on computed tomography (CT) scans of heavy drinkers and alcoholics (Cala et al. 1978; Carlen et al. 1978; Ron 1983). Brain shrinkage has also been demonstrated in "social" drinkers (Cala 1983). CT scans quantify this atrophy by measuring the ventricular volume and the sulcal, interhemispheric, Sylvian fissure, and forceps major widths (Fox et al. 1976; Ron 1983).

Pathological confirmation of this shrinkage came with studies of brain weight in alcoholic populations (Harper and Blumbergs 1982; Torvik et al. 1982; Lindboe and Loberg 1988). The mean reduction in brain weight in these studies ranged from 31 to 71 grams (Torvik et al. 1982; Harper and Blumbergs 1982). The Scandinavian research groups examined their data in relation to age and found no difference in brain weight between controls and alcoholics after 70 years of age (Torvik et al. 1982; Lindboe and Loberg 1988). However, the Scandinavians selected their subjects differently than the Australians in that WKS cases comprised 12.5 percent of the Scandinavian subjects (Torvik et al. 1982), compared with 50 percent of the Australian subjects (Harper and Blumbergs 1982). This probably explains the more severe brain weight and volume changes in the Australian study.

Table 1 suggests a graduated effect of alcohol exposure on brain weight in different drinking groups, the least severe being in moderate drinkers. On the other hand, alcoholics with additional complications, such as cirrhosis of the

TABLE 1

Fresh brain weight

	n	Weight (g)	SEM[1]
Control	56	1,433	17
Moderate	16	1,415	34
Uncomplicated alcoholic	38	1,352[2]	27
Alcoholic + cirrhosis	21	1,321[2]	19
Alcoholic + WKS	23	1,310[3]	41

Notes: [1]*SEM = standard error of the mean.*
[2]*P < 0.01*
[3]*P < 0.001*

liver or WKS, have the greatest loss of brain tissue.

Brain volumes show similar patterns of change in alcoholics. However, no significant differences exist between males and females, although both groups are significantly different from the controls.

Data on the weights of major individual components of the brain are also available. Table 2 lists the weights of the left cerebral hemisphere and cerebellum in different groups of male alcoholics. No differences have been identified in comparisons of right and left hemispheres (Harper et al. 1985), although other studies have suggested that the right and left hemispheres are differentially susceptible to the effects of alcohol (Golden et al. 1981).

PERICEREBRAL SPACE

Brain weight and volume exhibit a wide range of variation, even in the normal population. A more reliable parameter to detect alcoholic brain shrinkage (or any change in an individual's brain volume) is

TABLE 2

Cerebral hemisphere and cerebellum weights

	n	Cerebral hemisphere Weight (g)	SEM	Cerebellum Weight (g)	SEM
Control	34	621	54	186	15
Moderate	12	621	60	186	20
Uncomplicated alcoholic	18	592	63	180	18
Alcoholic + cirrhosis	13	566[1]	36	168[2]	16
Alcoholic + WKS	9	549[1]	43	162[1]	15

Notes: [1]*P < 0.001*
[2]*P < 0.01*

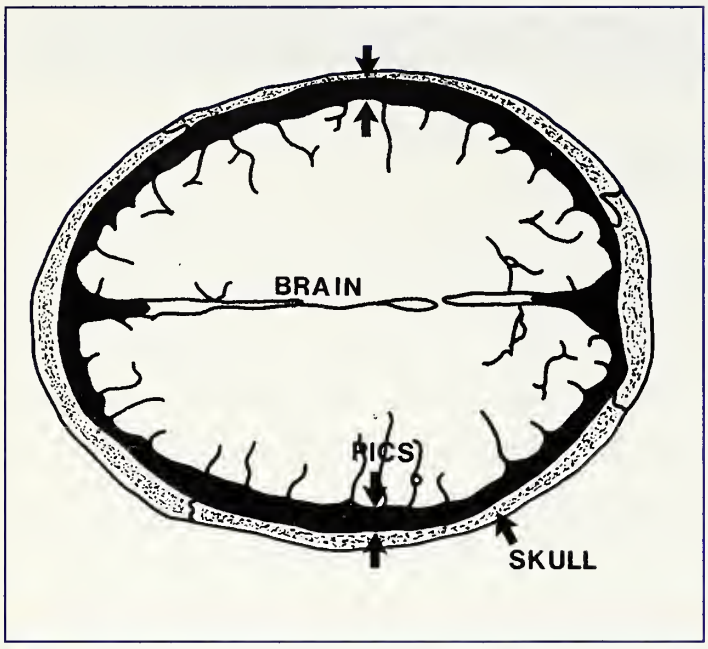

FIGURE 1

Diagrammatic representation of horizontal section through the skull and brain showing PICS space (colored black). Reproduced with permission from Harper and Kril (1985).

the measurement of pericerebral space (PICS) (figure 1). The usefulness of the PICS measurement relates to the fact that during childhood the growth of the brain influences the growth of the skull (Dekaban and Sadowsky 1978). The intracranial cavity volume (ICV) then remains unchanged during adult life (Davis and Wright 1977). Any reduction in brain volume will cause an increase in the PICS value, which can be calculated from the following equation:

$$\text{PICS} = \frac{\text{ICV} - \text{Brain Volume}}{\text{ICV}} \times \frac{100\%}{1}$$

There are very few large studies of these parameters in either normal populations or pathological states. This is because of the logistic and mechanical difficulties of obtaining these measurements during routine necropsies. Techniques for measuring ICV have varied from filling the cavity with sand or ball bearings (Reichardt 1905; Hoff and Seitelberger 1957) to the inflation of a balloon with water inside the cranial vault (Davis and Wright 1977). The most simple and reliable technique for the measurement of ICV was described by Harper et al. (1984). This technique involves the production of a permanent polyurethane cast of the cranial cavity. These can be formed in about 20 minutes with little modification of standard necropsy technique. Using this method it has been shown that alcoholics (mixed male and female group) have a mean PICS of 11.3 percent, compared with a control value of 8.3 percent (Harper and Kril 1985). In a similar recent study of female alcoholics (Harper et al. 1990), the PICS value was 16.3 percent, compared with 9.5 percent in female controls. Moderate drinkers had a mean PICS value of 9.8 percent, but the difference from controls did not attain statistical significance (Harper et al. 1988*a*). There is a reasonable correlation between the subjective assessment of cortical atrophy and ventricular dilatation and high PICS values. The highest PICS values were seen in alcoholic subjects who had WKS (14.7 percent) and cirrhosis of the liver (16.2 percent) (Harper and Kril 1985). Figure 2 shows the PICS value for each of the alcoholic groups.

VOLUME CHANGES WITHIN THE CEREBRAL HEMISPHERES

Macroscopic examination of the cerebral hemispheres of alcoholic patients has been

TABLE 3

Percentage grey matter and white matter

	n	Grey matter Volume (%)	SEM	White matter Volume (%)	SEM
Control	26	53.8	0.6	40.5	0.5
Moderate	13	55.3	0.7	39.4	0.7
Uncomplicated alcoholic	34	54.3	0.5	39.1[1]	0.4
Alcoholic + cirrhosis	18	55.8[2]	0.7	37.8[1]	0.8
Alcoholic + WKS	15	57.2[1]	0.9	36.4[3]	0.7

Notes: [1]$P < 0.01$
[2]$P < 0.05$
[3]$P < 0.001$

generally considered unrewarding apart from the frequent finding of old head injuries (Harper 1979; Skullerud et al. 1991) and specific associated diseases such as WKS (Victor et al. 1971). Courville (1955) and Lynch (1960) have commented on the apparent regional nature of the cortical atrophy in that the frontal lobes seem more severely affected. However, no specific quantitative studies of alcoholic cerebral atrophy had been done until recently. The aim of these studies was to identify specific regional changes of brain tissue loss to explain cerebral shrinkage. After fixation in formalin, brains were embedded in agarose and sectioned in the coronal plane at 3-mm intervals. Photographs were prepared of each brain slice, and morphometric analyses were carried out to measure the volumes of the cortical grey matter, white matter, "basal ganglia" (which included thalamus, hypothalamus, and basal ganglia), and ventricles (Harper et al. 1985). The volumes of grey and white matter in the alcoholic and control groups are presented in table 3 and are expressed as a percentage of the cerebral hemisphere volume.

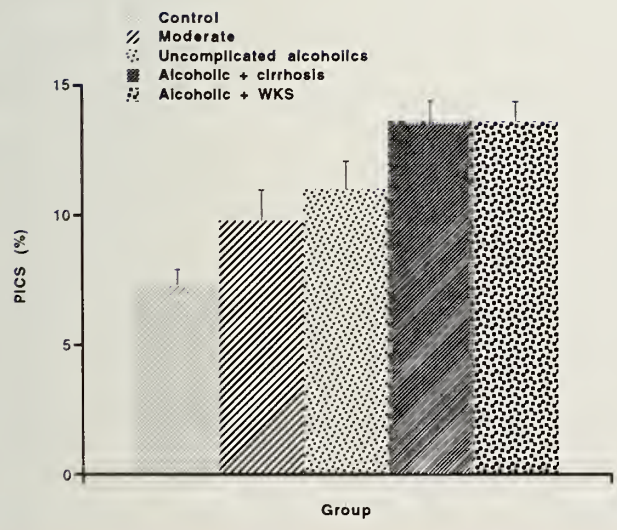

FIGURE 2

Bar graph showing the progressive increase in PICS value (brain shrinkage) for moderate drinkers and the three alcoholic groups (mean ± SEM).

WHITE MATTER

The most interesting findings from these studies were significant reductions in the volume of the white matter of the cerebral hemispheres in uncomplicated alcoholics and alcoholics with WKS and cirrhosis.

There was a significant age effect in the control and alcoholic groups. In another study of alcoholic subjects, de la Monte (1988) used morphometric techniques and found reductions of 6.1 to 17.5 percent in the volume of cerebral white matter. Both Harper et al. (1985) and de la Monte (1988) showed an absolute increase in the the size of the ventricles, the latter author concluding that the increase in the size of the ventricles was roughly equal to the amount of white matter loss.

Many imaging studies of alcoholics have shown that the frontal lobes are more shrunken than other brain regions (Jernigan et al. 1991*a*,*b*). One reason these frontal lobe changes are more evident is the greater proportion of white matter compared with cortical grey matter in frontal regions. The proportion of grey to white matter is 1.22 in the frontal region and 1.40 in the occipital lobes (Harper et al. 1985). However, there does not seem to be a differential susceptibility of the frontal lobes as discussed in the cerebral cortex section. The volume of white matter in the cerebellar vermis is also reduced in uncomplicated alcoholics when compared with controls (Phillips et al. 1987). Those alcoholics with WKS showed a greater reduction in white matter compared with uncomplicated alcoholics.

Specific white matter structures have also been measured in the brains of alcoholic patients. The corpus callosum is significantly reduced in thickness in alcoholics when compared with age- and sex-matched controls (Harper and Kril 1988). In addition, there was a significant linear correlation between corpus callosum thickness and white matter volume in the alcoholics. Although this study was carried out on postmortem material, the corpus callosum can be well visualized and measured using MRI scans (Nasrallah et al. 1986). Thus, corpus callosal thickness could be used as an in vivo diagnostic parameter to assess brain shrinkage in alcoholics. Measurements could also be done sequentially to identify and quantitate the reversibility of the brain shrinkage, which has been well documented in many abstinent alcoholics (Carlen et al. 1978).

At the light microscopic level, the white matter in the cerebral hemispheres of alcoholic subjects was said by Courville (1955) to show "alterations in the structure and a decrease in the number of the nerve fibres . . . ." He stated that the most conspicuous changes were observed in myelin-stained sections and that the radiating fibers of the crowns of cerebral gyri were most affected.

Other authors have reported diffuse pallor of white matter in myelin-stained preparations, particularly in cases of WKS (Cravioto et al. 1961). The almost total loss of myelinated fibers in the mammillary bodies of WKS cases has been demonstrated by several authors (Pollock 1974; Victor et al. 1989). Alling and Bostrom (1980) showed similar changes in nine chronic alcoholic subjects without WKS and further analyzed the mammillary body tissue chemically. They found significantly lower concentrations of phospholipids, cholesterol, and cerebroside, implying a loss of myelin in the alcoholic cases.

Witt (1985) reviewed the neuroanatomical consequences of thiamine deficiency (WKS), comparing human material with animal models. She concluded that pallor of myelin was seen in acute WKS cases, and total loss of myelin suggested chronic changes.

Ultrastructural studies in animal models of WKS show that the predominant early lesions are intracellular edema of astrocytes, oligodendrocytes, and myelin sheaths (Robertson et al. 1968; Watanabe and Kanabe 1978). There is actual destruction of myelinated fibers in more advanced lesions (Dreyfus and Victor 1961). Blank et al. (1975), in a study of monkeys subjected to varying lengths of thiamine deficiency, reported the primary structural alterations to be myelin "blister" formations. However, myelin loss was not observed in a similar animal model examined by Witt and Goldman-Rakic (1983*a*,*b*).

Ultrastructural studies of the effects of alcohol alone on the structure of CNS myelin in an experimental model have been performed (Phillips 1989; Phillips et al. 1991). Alcohol exposure causes a reduction in the relative thickness of myelin sheaths in rat optic nerves. Similar studies using human material have not been possible because of the poor preservation of myelin sheaths after death.

The above discussion implies that white matter changes in the brains of alcoholic subjects, particularly those with WKS, are obvious when sections of brain are stained appropriately and examined under the microscope. However, this is not so. We have recently reviewed the myelin-stained sections (Loyez technique) of frontal, temporal, parietal, and occipital cortical regions of 20 controls, uncomplicated alcoholics, and alcoholics with WKS. There are significant variations in the pattern of myelinated fibers at the crowns of the gyri in different cortical regions (least fibers seen in the temporal cortex), but we were unable to identify consistent differences among the three groups of cases. Nevertheless, further studies may be warranted using more objective measuring techniques, such as automated densitometry.

The subtle nature of the white matter changes is borne out by physical and chemical studies of the white matter of alcoholic subjects. The specific gravity of the frontal, parietal, and occipital white matter in alcoholics and age- and sex-matched controls has been measured. There was no significant difference in the specific gravity, indicating no difference in white matter density in the alcoholic group as a whole. However, there was a slight reduction in those alcoholics with cirrhosis of the liver (Harper et al. 1987*a*). In the alcoholic group as a whole, there was a slight increase in the water content of the white matter. This increase only attained statistical significance in the frontal lobe of those alcoholics with WKS (Harper et al. 1987*a*, 1988*b*). Nevertheless, this change in hydration in alcoholic subjects confirms in vivo studies using magnetic resonance imaging (MRI) (Besson et al. 1981; Smith et al. 1985) and refutes the hypothesis that changes in hydration can account for cerebral shrinkage and the reversible cerebral shrinkage seen in alcoholics.

Studies of the lipid profiles of the white matter in the different alcoholic groups have shown only minor alterations (Lesch et al. 1972; Harper et al. 1988*a*, 1989). The most interesting feature is that the water content rises and the lipid content falls in the alcoholic groups, whereas the reverse occurs in the moderate group (Harper et al. 1988*a*,*b*). How might these results be explained on anatomical or pathological grounds?

White matter is composed of approximately 70 percent water, 20 percent lipids, and 10 percent protein (O'Brien and Sampson 1965). As most of the lipid and protein is combined to form membranes (myelin in particular), if there is an increase in lipid content, there will be a decrease in water content and vice versa. Neurochemical studies of the white matter from demyelinating lesions show an increase in water content and a decrease in lipid content (Davidson and Wajda 1962). However, the magnitude of the change is much greater in demyelinating lesions than in moderate drinkers. A similar pattern is seen in Wallerian degeneration (Cumings and Kremer 1965), although most of these studies have used experimental animal models.

During maturation of the brain, there is active myelination and the patterns are reversed: a decrease in water content and an increase in lipid content (O'Brien and Sampson 1965). The lipid and water changes noted in the moderate and uncomplicated alcoholic groups may, therefore, be an indication of some subtle structural change in the white matter. Perhaps the rat model of Phillips et al. (1991) provides the pathological explanation. In the alcohol-treated animals, myelin sheaths appear normal but have fewer lamellae than equivalent control material. Such a change would result in a slight increase in water content and little variation in the protein, lipid, and carbohydrate profiles of the white matter. Nevertheless, these changes could induce important functional abnormalities.

CT scans can be used to measure the density of cerebral white matter during life (Baldy et al. 1986). However, results obtained from such studies of alcoholic populations are contradictory. Golden et al. (1981) reported a decreased density of the left hemisphere in alcoholics, whereas Cala (1982) found no difference between control and alcoholic populations. Lishman et al. (1987) have reported more detailed studies of the density of the cerebral white matter in alcoholics. They showed that the x-ray absorption density of the white matter rose slightly as the duration of abstinence increased. MRI showed that one-half of an uncomplicated alcoholic group had multiple round hyperintense areas (Gallucci et al. 1989). The authors suggest that changes in white matter occur in a significant proportion of asymptomatic uncomplicated alcoholic patients.

Two other specific white matter disorders have been described in alcoholic subjects: central pontine myelinolysis (CPM), which is discussed under the heading "brain stem," and Marchiafava-Bignami (MFB) disease. The latter is an extremely rare condition. Despite our particular interest in the neuropathology

of alcohol-related disorders, we have only seen one case in 10 years (approximately 10,000 brains). The incidence seems to be much higher in France, where 17 cases of MFB disease were diagnosed from 8,200 necropsies, a prevalence of 0.21 percent (Hauw et al. 1988). Nine of the cases were associated with pellagra-like changes in the central nervous system. In the same study, the prevalence of WKS was 1.35 percent, compared with 1.7 and 2.8 percent from two similar studies (Cravioto et al. 1961; Harper 1983).

MFB disease is characterized by symmetrical degeneration of myelin and, to a lesser extent, of axons in the central portion of the corpus callosum. The histological features in the affected regions resemble those seen in central pontine myelinolysis. There are several reports in which the two diseases were associated (Ghatak et al. 1978), providing circumstantial evidence of a common pathogenetic mechanism.

CEREBRAL CORTEX

Although most of the tissue loss from the cerebral hemispheres in alcoholics can be explained by a reduction in the volume of cerebral white matter, there is also generally a slight reduction in the volume of the cerebral cortex. This has been demonstrated both pathologically (de la Monte 1988) and using MRI with quantitative morphometry (Jernigan et al. 1991*a*). Not all the alcoholic groups have reduced cortical grey matter. Although in many alcoholic cases an apparent atrophy of the cerebral cortex occurs with widening of cortical sulci and narrowing of gyri, this could be explained based on loss of white matter as discussed above. Measurements of cortical thickness have been rarely reported, but Mayes et al. (1988) noted a reduction in the frontal region in one carefully studied case of Korsakoff's psychosis.

At the microscopic level, several authors have subjectively described a patchy loss of cortical neurons in alcoholics (Courville 1955; Victor et al. 1971). The first quantitative study documenting neuronal loss in alcoholics was published in 1987 (Harper et al. 1987*b*). There was a 22-percent reduction in the number of neurons in the superior frontal cortex (Brodmann's area 8), but no significant change in the primary motor (area 4), frontal cingulate (area 32), or inferior temporal (areas 20 and 36) cortices (Kril and Harper 1989). Mayes et al. (1988) identified neuronal loss from the gyrus rectus (inferior frontal) in one of the two cases of Korsakoff's psychosis studied. Shrinkage of cortical neurons was also shown in the superior frontal, motor, and frontal cingulate cortices (Harper et al. 1987*b*; Kril and Harper 1989).

These findings of severe damage to the frontal cortex in alcoholics are consistent with clinical (Walsh 1985) and neuroradiological studies (Jernigan et al. 1991*a*) that suggest the frontal lobe may be more susceptible to alcohol-related brain damage than other cortical regions. Moreover, particular groups of neurons may be more likely to be damaged. An analysis of the pattern of neuronal loss from the superior frontal cortex in alcoholics revealed that large pyramidal neurons with a somal area

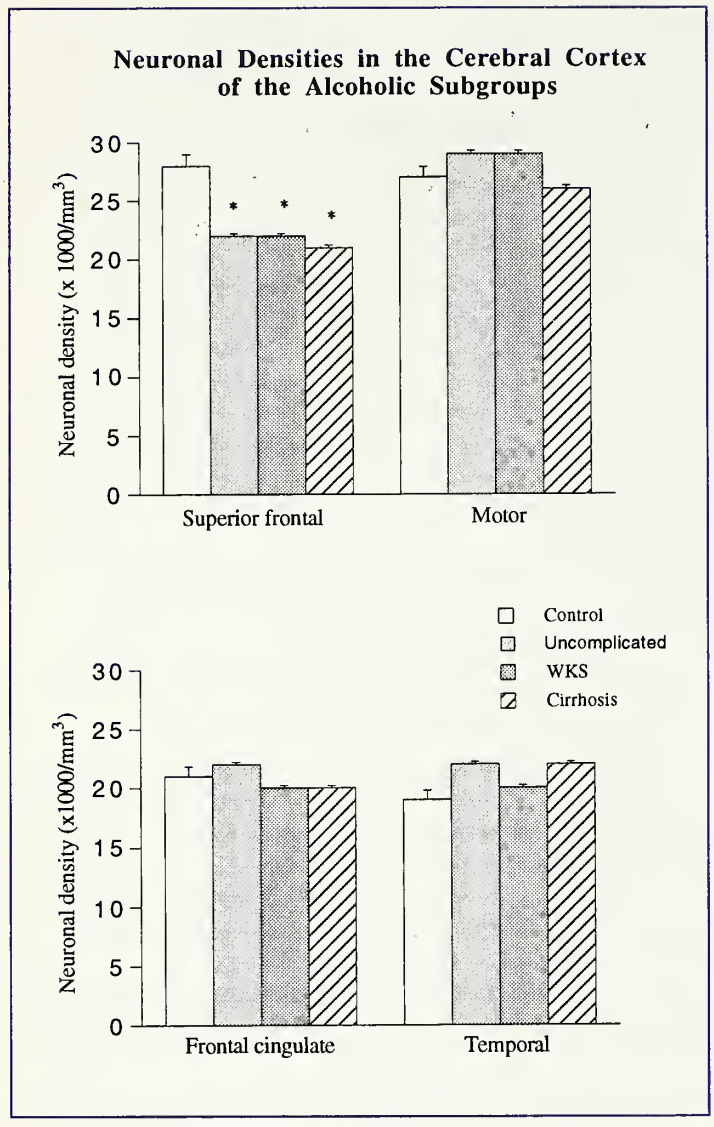

FIGURE 3

Neuronal counts from four cortical regions in controls, uncomplicated alcoholics, alcoholics with cirrhosis of the liver, and alcoholics with WKS. There was a significant (*) loss of neurons from the superior frontal cortex in all the alcoholic groups.

greater than 90 μm^2 were selectively lost (Harper and Kril 1989). This population of large neurons has been recognized as more vulnerable in both Alzheimer's disease (Terry et al. 1981) and the normal aging process (Terry and Hansen 1987). There is no evidence to suggest that particular layers of the cerebral cortex are more vulnerable than others (Harper and Kril 1989).

Subpopulations of cortical neurons can now be identified by their neurochemical content using immunohistochemistry (Beal and Martin 1986). These techniques have yet to be applied to alcohol-related brain damage. There are some neurochemical and neuropharmacological data to suggest that these techniques might provide useful information (Tran et al. 1981; Freund and Ballinger 1988*a*,*b*).

Ultrastructural studies have not been possible using human material. However, Harper and Corbett (1990) have examined and measured the dendritic arbor of cortical neurons in alcoholic subjects using Golgi impregnation techniques. They showed a significant reduction in the basal dendritic arbor of layer III pyramidal neurons in both the superior frontal and motor cortices (Harper and Corbett 1990). This study suggests that although no significant reduction in numbers of cortical neurons in the motor cortex is found, there are cellular structural abnormalities that could have important functional implications.

Analysis of cortical neuronal counts in the different alcoholic groups revealed no significant difference between those alcoholics with WKS or cirrhosis of the liver and the uncomplicated alcoholics, as shown in figure 3 (Kril and Harper 1989). This finding suggests that alcohol abuse is responsible for the neuronal loss in the superior frontal cortex and that the additional complication of WKS, although significantly contributing to the brain shrinkage in alcoholics (table 3), does not accentuate neuronal loss.

One other cortical abnormality described in alcoholics is Morel's laminar sclerosis (Victor et al. 1989). This is

necrosis and degeneration of layers III and IV of the cerebral cortex. In two large pathological studies of WKS, no cases of Morel's sclerosis were identified. It is frequently associated with MFB disease and is thought to be secondary to disruption of fibers of the corpus callosum (Victor et al. 1989). However, it has been reported in isolation (Naeije et al. 1978).

HIPPOCAMPUS

The hippocampal formation lies in the medial segment of each temporal lobe in the human brain. This region has been poorly studied in alcoholic subjects despite the fact that pathological changes in the hippocampus have dominated the literature on experimental animal models of alcohol toxicity (Walker et al. 1980; McMullen et al. 1984). Walker et al. (chapter 11) discuss these changes.

Anatomically, the hippocampus in humans consists of five cytoarchitectural regions, which have a complex structural arrangement. Microscopically, there are several different neuronal populations within the hippocampus, some of which can be identified immunohistochemically by their neurochemical composition.

The main afferent projections to the hippocampus are from the brain stem (locus ceruleus, dorsal raphe, and tegmentum of pons), hypothalamus, thalamus, basal forebrain, amygdala, and some regions of neocortex. The efferent projections pass to the amygdala, prefrontal cortex, olfactory tubercle, thalamus, and hypothalamus. Some anatomical loci mentioned above (e.g., thalamus and hypothalamus) have already been shown to be abnormal in alcoholic subjects with WKS (Harper 1983; Torvik 1985; Victor et al. 1989).

Given that the hippocampal formation is integral to memory function and that memory deficits have been documented in both uncomplicated alcoholics (Ron 1983; Salmon and Butters 1987; Tuck and Jackson 1991) and alcoholics with WKS (Victor et al. 1989; Parkin et al. 1990), this region requires far more attention by neuropathologists. In most neuropathology departments, it is routine to examine the hippocampus macroscopically and microscopically. However, the hippocampal formation extends over about a 50-mm anteroposterior length, and a single section will provide only limited information. Moreover, as shown in the experimental animal studies of alcohol toxicity (Walker et al. 1980), careful quantitative analyses are required to identify the abnormalities.

BASAL GANGLIA

The basal ganglia consist of the caudate nucleus, the putamen, and the globus pallidus and are primarily involved in extrapyramidal motor functions. The basal ganglia lie close to the thalamus and hypothalamus and border the frontal horns of the lateral ventricles. This region has received little attention in alcoholics. Harper et al. (1985) in their analysis of brain shrinkage in alcoholics, measured the volume of the "basal ganglia" (including the thalamus and hypothalamus). They found no significant loss of tissue from this region. However, it should be noted that the point-counting technique

used in their study is not sensitive enough to detect subtle changes in the volume of a structure that constitutes only 5 percent of the total hemisphere volume.

Microscopic damage to the basal ganglia is not apparent in alcoholics, although no comprehensive quantitative studies have been published. Victor and colleagues (1989) did not comment on the involvement of the basal ganglia in their extensive examination of patients with WKS.

Recent studies, particularly in animal models of alcohol toxicity, have identified abnormalities in neurotransmitter receptor binding in the basal ganglia. One study using tissue from human alcoholics has identified a loss of muscarinic cholinergic receptors from the putamen in alcoholics, but found no change in the benzodiazepine receptors (Freund and Ballinger 1989). There is no clinical evidence that the functions of the basal ganglia are impaired in either uncomplicated alcoholics or alcoholics with WKS.

THALAMUS

The thalamus is the principal relay station between the cortex and the periphery. It integrates sensory and motor functions and plays important roles in arousal, consciousness, and sleep. The anterior and medial dorsal nuclei of the thalamus are thought to be important components of memory pathways. Ohye (1990) has given a detailed review of the complicated anatomical subdivision of thalamic nuclei and their proposed functions.

In uncomplicated alcoholics there is a total lack of information concerning pathological changes in thalamic nuclei. There are no obvious specific macroscopic or microscopic abnormalities, based on our routine examination of the thalamic nuclei in more than 100 uncomplicated alcoholic cases. Nevertheless, as has been shown in various neuropathological disorders, significant changes in total neuronal counts, neuronal density, and mean neuronal size can easily be overlooked unless detailed quantitative studies are carried out. The only experimental study of the effects of alcohol on thalamic nuclei was published by Berachochea et al. (1987). They showed a significant decrease in the volumes of the medial dorsal and anterior nuclei of the thalamus in mice that had consumed alcohol for 6 to 7 months.

The thalamus appears to be particularly susceptible to damage in WKS. Table 4 lists the frequency of thalamic lesions in five of the largest pathological studies of WKS.

The wide range between these studies (53 to 100 percent) may relate to methods of case selection and the detail in which the cases were examined. In the study by Victor et al. (1971), most of the cases had been diagnosed as WKS before death. In the study by Harper (1983), only 20 percent of the cases had been diagnosed prior to death, and many cases had none or only one of the classical clinical signs of WKS (Harper et al. 1986). Thus, the pathological changes in this latter group might be expected to be less severe.

From a pathological point of view, the most detailed study was that of Victor et al. (1971, 1989), who examined serial sections of the thalamus in 17 cases and multiple representative sections in anoth-

TABLE 4

Incidence of thalamic lesions in pathological studies of WKS

	Year	n	Thalamic Lesions (%)
Malamud and Skillicorn	1956	70	53
Cravioto et al.	1961	28	100
Victor et al.	1971	45	89
Harper	1983	131	61
Torvik	1985	46	69

er 23 cases. They listed the incidence of involvement in 24 different thalamic nuclei. The most frequently affected nuclei were the medial dorsal (88 percent), submedius and medial ventral (58 percent), lateral dorsal (68 percent), and medial pulvinar (85 percent). Torvik (1985) studied 46 cases of WKS, but only 21 had step sections through the thalamus. The investigator only commented on four different regions: the medial dorsal nucleus, the medial part of lateral nucleus, the anterior nucleus, and the pulvinar. The medial dorsal nucleus was by far the most commonly affected region (76 percent).

Both studies used subjective criteria in their evaluation, and Victor et al. (1989) commented that "some degree of variation in the incidence of involvement must be dismissed as an error in technique." It must be emphasized that it is often extremely difficult to decide whether there are neuronal loss and gliosis in the thalamus. More sophisticated neuronal and glial markers (e.g., glial fibrillary acid protein and antibodies to neurochemicals) and quantitative morphometry are now essential tools in such studies.

The distribution of lesions in the thalamus is an important issue. Most authors believe that the severe amnestic syndrome that characterizes Korsakoff's psychosis is caused by lesions in specific thalamic and hypothalamic regions (Malamud and Skillicorn 1956; Victor et al. 1971; Mair et al. 1979; Mayes et al. 1988). However, there is some debate as to the most relevant thalamic nuclei. Damage to the medial dorsal nucleus was first correlated with the amnesia by Victor et al. (1971). Animal models of WKS support these findings in that degeneration of the medial dorsal thalamic nucleus correlates with a loss of spontaneous synchronous bursts of activity in the cortex (Armstrong-James et al. 1988).

However, detailed studies of four autopsied cases of alcoholics with Korsakoff's psychosis have shown damage to the medial thalamus, but the medial dorsal nucleus was largely spared (Mair et al. 1979; Mayes et al. 1988). A thin band of gliosis between the wall of the third ventricle and the medial magnocellular portion of the medial dorsal nucleus was noted. Mair et al. (1979) concluded that lesions in this site (paratenial nucleus) can

FIGURE 4

Coronal slice of the cerebral hemispheres at the level of the mammillary bodies. There is symmetrical bilateral periventricular necrosis (arrows), which is occasionally seen in acute WKS.

induce memory impairment. This nucleus borders the medial dorsal nucleus, and Victor et al. (1989) criticized the work of Mair et al. (1979), stating that gliosis extended into the medial dorsal nucleus.

Thalamic lesions have been reported in WKS alcoholics without amnesia (Malamud and Skillicorn 1956; Torvik 1985). The lesions are similar to those seen in Korsakoff's psychosis but differ from those seen in other brain regions (e.g., mammillary bodies) in the same cases. The principal difference is an almost complete loss of neurons in the thalamus, compared with relative sparing of neurons elsewhere (Torvik 1985). In rare cases of WKS, symmetrical zones of necrosis are seen in the thalamus (figure 4). Such lesions are similar to those seen in experimental models of WKS in which thiamine antagonists (e.g., pyrithiamine) are used to induce the disorder.

In spite of these studies, there are still important unanswered questions. The principal question is the definitive identification of the anatomical substrate of the severe amnesia in Korsakoff's psychosis. Morphometric studies will answer this question on well-documented cases of WKS.

HYPOTHALAMUS

The hypothalamus is a very small anatomical region that contains the integrative systems for such activities as fluid and electrolyte balance, thermoregulation, appetite, immune and emotional responses, and reproduction. Most neuropathologists examine the hypothalamus both macroscopically and microscopically in cases of alcoholism. No pathological abnormalities have been described, except in cases with additional WKS. Nevertheless, quantitative morphometric studies that may identify these abnormalities have not been reported. With improved in vivo imaging techniques, lesions in anatomical structures as small as the mammillary bodies can now be identified (Charness and DeLaPaz 1987). In a recent MRI study (Jernigan et al. 1991*b*), a reduction in the volume of the anterior diencephalon in eight alcoholics with Korsakoff's psychosis was found. This region included septal nuclei and anterior hypothalamic grey matter. There are no equivalent pathological studies, perhaps because of the difficulty of identifying and measuring these specific regions within the hypothalamus.

The hypothalamus is the most consistently involved part of the brain in WKS. In studies by Victor et al. (1971) and Harper (1983), abnormalities were noted

in 96 and 99 percent of cases, respectively. Both studies indicate that the mammillary bodies are the central focus of the disease. Victor et al. (1971) list the prevalence of involvement of all hypothalamic nuclei in WKS and give detailed descriptions of pathological changes.

Macroscopically, the changes seen depend upon the stage of the disease. Cases can be readily classified as acute, chronic, or acute-on-chronic. In a pathological study of 131 cases of WKS, 17 percent were acute, 66 percent chronic, and 17 percent acute-on-chronic (Harper 1983). In a Scandinavian study of 45 cases, 53 percent were acute and 47 percent chronic (Torvik 1987).

Without visible hemorrhages, which are only seen in about 5 percent of cases, it is possible to miss the diagnosis of acute WKS unless sections are taken and examined microscopically. Twenty-five percent of cases can have normal mammillary bodies on macroscopic examination (Harper 1983). The most consistent abnormality in chronic WKS is shrinkage and brown discoloration of the mammillary bodies (figure 5). Unilateral mammillary body changes have been reported but are much more likely to be caused by posterior cerebral artery territory infarction or transynaptic degeneration following an ipsilateral hippocampal lesion.

Microscopic changes can be related to the duration of the disease up to 10 to 14 days. The earliest changes are seen in the neuropil and in and around blood vessel walls. There are edema and extravasation of red blood cells into the perivascular spaces. Sometimes these extend outward into the parenchyma to form "ball" microhemorrhages or macroscopically visible hemorrhages. Within 1 to 2 days the endothelial cells become hypertrophic and capillary budding commences. These changes are maximal at 7 to 10 days. Tissue necrosis is occasionally seen but is more common in the thalami. Neurons show relatively little change and do not appear to be the principal targets of this disease process. There is relative sparing of neurons even in chronic WKS. Thalamic and olivary neurons seem an exception to this rule. An astrocytic reaction is noted by the third or fourth day. Myelin and axons are often destroyed. Apart from occasional histiocytes, there is generally no inflammatory reaction.

In chronic lesions, parenchymal elements are lost and reactive changes are largely restricted to increased numbers of astrocytes. There are increased numbers of thin-walled capillaries with normal

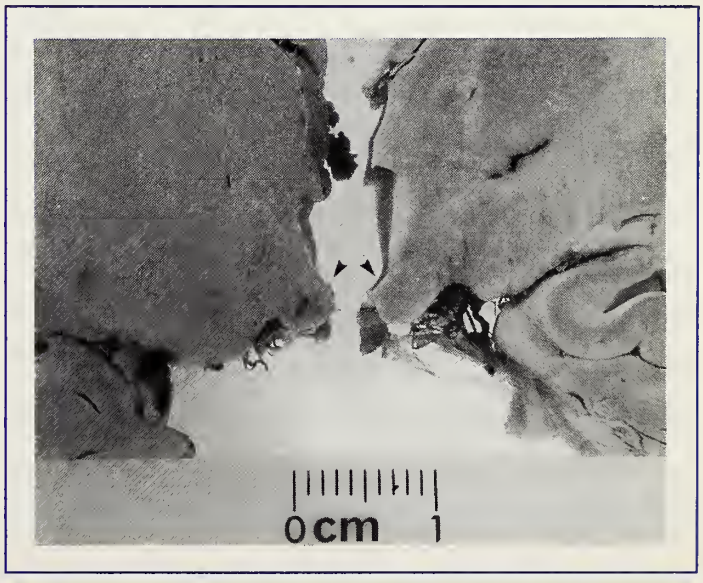

FIGURE 5

Hemi-coronal slices at the level of the mammillary bodies from two different cases. The right side is normal. The left mammillary body is small and has a central spongiotic appearance typical of chronic WKS.

endothelium. This is partly due to a loss of parenchymal elements and a compaction of surviving elements and partly reflects the vascular proliferation that occurs in the acute stage. There is a relative preservation of neurons but a loss of myelin and axons. Hemosiderin-laden macrophages in perivascular spaces are the tombstones of microhemorrhages. In the most severe cases the mammillary bodies become shrunken and spongy with almost no residual parenchyma (figure 5).

Changes in other hypothalamic nuclei display a similar pattern of disease, but the changes are usually much less severe and can be quite difficult to identify in chronic WKS.

BASAL FOREBRAIN

The cholinergic basal forebrain nuclei are found within the medial septal nucleus, the nucleus of the diagonal band, the basal nucleus of Meynert (NbM), and the substantia innominata (Mesulam and Geula 1988). These nuclei project to different regions of the brain (Butcher and Semba 1989). Broadly speaking, the medial septal nuclei project to the hippocampus; the diagonal band projects to the hippocampus, olfactory bulb, and limbic cortex; the substantia innominata projects to the amygdala and limbic cortex; and the NbM projects to the remaining cerebral cortex. The latter nucleus provides the major source of cholinergic innervation to the cerebral cortex.

Neuropsychological studies have suggested that the basal forebrain region is damaged in chronic alcoholics (Divac 1975; Jones et al. 1976). Supportive evidence for pathological changes in the basal forebrain has been noted in animal studies (Arendt et al. 1988). Chronic intake of alcohol in rats results in a decrease in the number of basal forebrain cholinergic neurons and a depletion of cortical choline acetyltransferase. These changes correlate with impairments in learning and memory function. Moreover, cholinergic-rich transplants appear to reverse the memory deficits in alcoholic rats (Arendt et al. 1988; also see Arendt, chapter 22).

There is controversy as to the amount of damage to these regions in alcoholic subjects. Most pathological studies have focused on those alcoholics with dementia or amnesia (Korsakoff's psychosis) because of the documented abnormalities in the NbM of patients with Alzheimer's disease. Arendt et al. (1983) compared the pathological changes in the NbM in Alzheimer's disease, Parkinson's disease, and Korsakoff's psychosis. They calculated numbers of neurons and the neuronal population density from serial sections of the NbM. There was a significant loss of neurons in Alzheimer's disease (30 percent), Parkinson's disease (23 percent), and Korsakoff's psychosis (53 percent). They also studied five alcoholics without dementia, and the mean neuronal counts were no different from control data (Arendt et al. 1983).

Mayes et al. (1988) studied the NbM in two cases of Korsakoff's psychosis using morphometric methods. They were unable to show a reduction in neuronal numbers or nucleolar volume, but did comment that many neurons were shrunken and showed a

loss of Nissl substance. In this study, single sections of the NbM were taken as representative of this complex anatomical structure. This procedural difference could account for the conflicting results.

A study by Harper et al. (1991) using serial sections and three-dimensional reconstructions of the NbM in a single uncomplicated alcoholic has confirmed the findings of Arendt et al. (1983). There was a greater than 50-percent shrinkage of the NbM and a 20-percent loss of magnocellular neurons. Moreover, there was an increased density due to the overall shrinkage. The neuronal loss was principally from the anterior and posterior pole regions. This specific pattern of neuronal loss highlights the importance of adequate sampling of the NbM.

BRAIN STEM

In uncomplicated alcoholics, there have been no specific abnormalities noted macroscopically in the midbrain or medulla. An important disorder that affects the pons is central pontine myelinolysis, discussed in detail below. Other nuclei that have attracted attention recently are those with significant cortical projections, particularly the locus ceruleus and raphe nuclei.

The topography of lesions in WKS generally follows the pattern seen elsewhere in the brain. They are found in the periventricular and periaqueductal regions, except for olivary lesions. The pathological changes will depend upon whether the disease is acute or chronic.

The incidence of involvement of different nuclei and regions in the brain stem was presented in detail in the monograph by Victor et al. (1989). The most common sites of involvement were the central superior (70.4 percent), medial vestibular nuclei (71.1 percent), interpositus and prepositus (68.2 percent), and locus ceruleus (67.9 percent). Data for many other specific nuclei are given in their monograph.

Most other authors give figures for more general regions, such as midbrain, pons, and medulla, and highlight specific clinically relevant regions such as oculomotor and vestibular nuclei (Malamud and Skillicorn 1956; Cravioto et al. 1961; Harper 1979). Torvik (1987) studied 21 cases of WKS by step sections through the brain stem and noted that lesions were far more common in acute than in chronic cases of WKS. This is consistent with the clinical observations that ophthalmoplegia and nystagmus are usually quickly reversed in acute WKS after treatment with parenteral thiamine (Phillips et al. 1952).

Several authors have noted that those changes in the inferior olives, which are distant from the periventricular tissues, are common and morphologically different (Malamud and Skillicorn 1956; Torvik 1987). The change is characterized by neuronal loss with relative sparing of the neuropil and capillary endothelium (Torvik 1987). The change has been likened to that in the thalamus, as discussed above. To complicate the issue, the olives often show secondary changes resulting from alcoholic cerebellar vermal degeneration. This loss is presumed to be a transynaptic degeneration.

Little work has been done on brain stem changes in WKS using modern tech-

nologies. Details of studies on the locus ceruleus and raphe nuclei are outlined below, but other regions that should be targeted include the nucleus and tractus solitarius and ambiguus. These regions are of particular interest because of their role in cardiorespiratory control. A feature that has tended to be overlooked in cases of WKS is the frequency with which patients die suddenly and unexpectedly. In one study by Harper (1979), 32 of 51 WKS cases died in this way, and in 10 instances there was no evident cause of death at necropsy. Eight of the cases were acute, and lesions involving vital cardiorespiratory centers in the brain stem could well have accounted for their sudden deaths (Harper 1979, 1980).

CENTRAL PONTINE MYELINOLYSIS (CPM)

CPM is a relatively uncommon disorder that was first described by Adams et al. (1959) in three alcoholic patients and one patient with malnutrition. Victor et al. (1971) found an incidence of 11.8 percent in their study of 34 cases of WKS, whereas Harper (1979) found CPM in only 4 percent of 51 cases. The expansive descriptions and figures by Adams et al. (1959) of the pathological changes in the central pontine regions have not been bettered. Although CPM has been described most commonly in alcoholics with WKS, many other nonalcoholic groups of patients can be affected. The most common groups are those with severe liver disease (especially postorthotropic liver transplant) (Estol et al. 1989), severe burns (McKee et al. 1988), malnutrition, anorexia, and severe electrolyte disorders (Klineschmidt-DeMasters and Norenberg 1982). Often, the most important factor in the causation of CPM may be a too-rapid correction of a profound hyponatremia (Messert et al. 1979; Norenberg et al. 1982). There are, however, several recent reports that question the validity of this mechanism (Papadakis et al. 1990; Bird et al. 1990). The diagnosis of CPM is being made more frequently with the availability of CT and MRI scans (DeWitt et al. 1984). Initially, patients with CPM were thought to have a very high mortality rate. However, MRI scans have permitted the identification of many more cases in the earliest stages of the disease, and cases of complete clinical recovery are now being reported (Charness and Diamond 1984; Wakui et al. 1991).

Pathologically, most cases of CPM have very similar topography. The lesions are usually in the center of the basis pontis (figure 6) and extend from just below the

FIGURE 6

Myelin-stained section of the mid pons showing a central zone of demyelination in the basis pontis. The features are typical of central pontine myelinolysis (CPM). (luxol fast blue, x 1).

midbrain rostrally through the upper two-thirds of the pons. The cross-sectional topography is often butterfly-shaped or triangular and is characteristically symmetrical about the midline rostro-caudal axis. The tegmentum of the pons is usually not involved, and there is usually a rim of normal white matter surrounding the central lesion. The diagnosis is not always evident on macroscopic examination of the brain stem. Goebel and Herman-Ben Zur (1972) reported 10 cases, 3 of which were only identified after microscopic examination. The central pontine region is prone to poor fixation and a chalky white central zone can often mimic CPM.

Microscopically, the CPM lesion is recognized easily on the myelin-stained sections in which a sharply demarcated area of pallor can be identified within the basis pontis (figure 6). Other microscopic changes depend upon the age of the lesion. In those cases with a short history, there is demyelination with preservation of axons. Usually, no inflammatory reaction is found, and neurons in the nuclei of the basis pontis are preserved. There may be reduced numbers of oligodendroglia. As the lesion progresses, some axons undergo degeneration, and axonal swellings and fragmentation are noted. The transverse pontocerebellar fibers are mainly affected, with the long rostro-caudal tracts being involved next. In the most severe lesions, the central zone can become completely necrotic, although this is uncommon. Macrophages appear after several days, their cytoplasm being filled with myelin debris as the disease progresses. The single most important feature that enables one to distinguish between a central pontine infarct and CPM is the preservation of the neurons in the nuclei pontis in the latter case.

Extrapontine myelinolytic lesions have been reported in approximately 10 percent of cases (Wright et al. 1979). These authors state that the common sites for extrapontine lesions are the striatum, thalamus, cerebellum, and cerebral white matter. In their study of CPM in 85 liver transplant patients, Estol et al. (1989) found 11 cases of CPM, and 4 of these had extrapontine myelinolysis of the lateral geniculate nuclei. There are relatively few pathological studies of extrapontine myelinolysis, and the frequency of occurrence of these lesions should become evident with the use of CT and MRI in clinically diagnosed cases.

Ultrastructural studies in human CPM have been limited by the quality of the preservation of necropsy material. Nevertheless, two studies have shown that the mechanism of myelinolysis consists of intramyelinitic splitting, vacuolization, and rupture of the myelin sheaths (Forno and Rivera 1975; Powers and McKeever 1976). These changes do not simulate other demyelinating diseases, such as multiple sclerosis, but are seen in toxic and metabolic conditions (Powers and McKeever 1976).

LOCUS CERULEUS

Lesions in the noradrenergic locus ceruleus are said to cause impairment of attention and information processing. Also, there are probably links with learning and memory, as suggested by experimental models (Squire 1987). Although

there are no studies of the brain stem region in uncomplicated alcoholics in the literature, several groups of workers have emphasized the importance of this nucleus and its noradrenergic pathways in alcoholics with WKS (Mayes et al. 1988; McEntee and Mair 1990). Victor et al. (1971) noted abnormalities in the locus ceruleus in 19 of the 28 cases studied (67.9 percent). They did not provide any details of the pathological changes, and no quantitation was included. Mayes et al. (1988) used quantitative techniques to study the loss of neurons in the locus ceruleus in two alcoholics with Korsakoff's psychosis. The patient with the more severe amnesia had a significant loss of neurons (19 percent).

McEntee and Mair (1990) have used biochemical studies to address the question of abnormalities of noradrenergic pathways. They have shown significant reductions of noradrenaline and its metabolites in the cerebrospinal fluid of WKS patients. Moreover, there is evidence of memory improvement with noradrenaline replacement therapy (McEntee and Mair 1990).

Halliday et al. (1992*b*) have recently reported a quantitative study of the locus ceruleus in four uncomplicated alcoholics and nine alcoholics with WKS. Four of these latter cases had the severe amnesia of Korsakoff's psychosis. The data were compared with five age-matched controls. There were no significant differences in the number, morphology, or distribution of pigmented locus ceruleus neurons between any of the groups analyzed. This study contradicts the previous accounts of substantial cellular damage to this region in any alcoholic and suggests that damage to the locus ceruleus is not responsible for the amnesia in WKS.

RAPHE NUCLEI

The median raphe nucleus is a midline structure in the caudal midbrain and rostral pons. The dorsal raphe nucleus lies dorsal to the median raphe in the ventral part of the central grey matter and is separated from it by the medial longitudinal fasciculus. Together, these two nuclei provide the primary source of serotonergic axons innervating large regions of the forebrain, particularly the cerebral cortex, the limbic system, and the hypothalamus (Tork and Hornung 1990). The anatomical location of the dorsal raphe nucleus and its ascending serotonergic fibers is such that it is likely to be damaged by the periventricular lesions that are characteristic of WKS.

A quantitative study of dorsal raphe neurons in two alcoholics with WKS found no loss or degenerative changes in the region (Mayes et al. 1988). Victor et al. (1971) did not specifically mention the raphe nuclei in their study of the pathology of WKS but noted that the "central grey matter" was involved in 55 percent of the 40 cases. Of the many other pathological studies of WKS, none have mentioned the raphe nuclei. Biochemical studies have shown abnormally low levels of serotonergic metabolites in the cerebrospinal fluid of alcoholics with WKS, and drug therapies enhancing serotonergic activity significantly improve memory performance in such patients (McEntee and Mair 1990).

Halliday et al. (1992*a*) have recently completed a quantitative morphometric study of the dorsal and median raphe nuclei in alcoholics. They used immunohistochemistry to identify the serotonergic neurons (Halliday et al. 1990). They found a significant reduction (50 percent) in the number of serotonergic neurons from both raphe nuclei in all of the alcoholic cases when compared with controls. The loss was particularly evident in the pons. Thus, it appears that the serotonergic system is disrupted in alcoholics but is no worse in alcoholics with WKS.

CEREBELLUM

Atrophy of the cerebellum is commonly associated with alcoholism, and is characterized clinically by ataxia and incoordination of the lower limbs (Victor et al. 1971). In a general hospital autopsy study, Victor and Laureno (1978) found an incidence of 4.1 percent in patients over 18 years old. The incidence among alcoholics is much greater. Torvik et al. (1982) reported that 26.8 percent of alcoholics and 38.6 percent of alcoholics with WKS had cerebellar atrophy. Both Harper (1983) and Victor et al. (1971) studied patients with WKS and found incidences of cerebellar atrophy of 32 and 36 percent, respectively. Cerebellar atrophy can also be identified radiologically during life. Cala et al. (1978) identified cerebellar atrophy in 63 percent of their alcoholic patients, but they found no correlation between the degree of atrophy and the degree of psychological impairment or clinical signs of cerebellar dysfunction.

Macroscopically, the neuropathological findings indicate a shrinkage of the folia, particularly the anterior superior vermis. Microscopically, there is a loss of Purkinje cells with proliferation of Bergmann glia. Quantitatively, there is a significant reduction in the number and size of Purkinje cells, which is most marked in the smaller rostral and caudal lobes of the vermis (lobes I-IV, IX, and X) (Torvik and Torp 1986; Phillips et al. 1987). In addition, there is a reduction in the volume of the molecular and medullary layers in the vermis (Phillips et al. 1987). The Purkinje cell loss and shrinkage are most marked in those alcoholics with WKS (Phillips et al. 1990).

Studies of the dendritic arborization of Purkinje cells using Golgi impregnation techniques have revealed reduced arbor in alcoholics (Ferrer et al. 1984) and in rats fed an alcohol-containing diet (Pentney 1982; Tavares and Paula-Barbosa 1983; Pentney, chapter 12).

Thiamine deficiency has been implicated in the etiology of cerebellar degeneration. Adams (1976) described a disease identical to alcoholic cerebellar degeneration in malnourished individuals without alcoholism. In addition, the clinical features of cerebellar degeneration can be reversed by the administration of thiamine, even in the presence of continued alcohol consumption (Victor et al. 1989).

SPINAL CORD

There are no pathological studies of cases purported to be alcoholic myelopathy. Sage et al. (1984) reported five well-nourished alcoholic patients who developed progressive myelopathy. Abstinence from alcohol halted the progression of the dis-

ease, but there was no improvement. Most of the previous reports of myelopathy in alcoholics had been linked to cases with severe cirrhosis and porto-caval shunts and had been called "shunt myelopathy" (Zieve et al. 1960; Liversedge and Rawson 1966). The five cases studied by Sage et al. (1984) did not have severe liver disease or shunts, which led the authors to suggest that alcoholic myelopathy without hepatic encephalopathy may be more common than realized. They suggested that the disease could be masked by other common neurologic complications of alcoholism, especially neuropathy. Until the pathological basis of this condition is clarified, it is difficult to speculate further. In vivo studies, particularly those using MRI, could help in the diagnosis and identification of the site of the lesion(s), and pathologists should try to study the spinal cord of alcoholics at necropsy more frequently.

A common pathological abnormality in the spinal cord of alcoholics is posterior column degeneration. This is best seen in myelin-stained sections of the cord and is an ascending Wallerian degeneration secondary to peripheral neuropathy. This is an extremely common clinical finding in alcoholics (Victor et al. 1989).

LIVER FAILURE (CIRRHOSIS)

Cirrhosis of the liver has been suggested as a potential cause of brain damage in alcoholics (see Tarter et al., chapter 21). As shown in table 5, we have examined the brains from patients with nonalcoholic cirrhosis and with alcoholic cirrhosis. Based on regional cerebral hemisphere volumes, no significant changes in the brains of the nonalcoholic cirrhotics were observed compared with controls. On the other hand, the alcoholic cirrhotics showed significant white matter shrinkage (table 5).

Although this is only a small sample of nonalcoholic cirrhotics, the data suggest that cirrhosis per se does not contribute significantly to brain shrinkage. Neuroradiological studies by Lee et al. (1979) support this contention; but Acker et al. (1982) found a significant correla-

TABLE 5

Brain weight and volume measurements in alcoholic and nonalcoholic patients with cirrhosis of the liver

	n	% Grey Matter	% White Matter
Control	56	54.2 (.4)	40.1 (.4)
Alcoholic cirrhosis	21	56.4[1] (.7)	37.1[2] (.7)
Nonalcoholic cirrhosis	4	54.8 (.7)	39.4 (.5)

Notes: SEMs in parentheses.
[1]$P < 0.01$
[2]$P < 0.001$

tion between the degree of liver damage and brain shrinkage.

In acute hepatic encephalopathy, severe cerebral edema can develop, which may be the cause of death. This is very unusual in alcoholic subjects. The more common pathologic finding in alcoholic hepatic encephalopathy is a change in the size and shape of astrocytic nuclei. These changes were first described by von Hosslin and Alzheimer (1912), and the cells are now called Alzheimer II astrocytes. The nuclei are large (about 1.5 times the normal size), often lobulated or clefted, with a central clear zone and margination of chromatin (Martin et al. 1987). They often have a nucleolar-like structure that is not seen in normal astrocytes. These changes in the astrocytes are not uniformly distributed throughout the central nervous system, and even in a single area some astrocytes appear normal and others abnormal. They are seen most easily in the deep layers of the cerebral cortex, hippocampus, basal ganglia, midbrain, pontine nuclei, and cerebellar dentate nuclei (Harper 1982). There seems to be a direct relationship between the severity of the cellular change and blood ammonia levels (Martin et al. 1987; also see Tarter et al., chapter 21, for discussion relevant to hepatic encephalopathy), a correlation substantiated in animal models (Pilbeam et al. 1983). Interestingly, the changes in the astrocytes are more pronounced following immersion fixation than perfusion fixation and may be due to postmortem fluid shifts, the dimension of the shift being determined by ammonia-induced translocation of ions (Pilbeam et al. 1983).

CONCLUSIONS

It is evident from the studies outlined in this chapter that the neuropathological changes seen in alcohol-related brain damage affect the cortex, white matter, diencephalon, brain stem, and spinal cord. The basal ganglia appear spared, but Jernigan et al. (1991*a*), using MRI, have now shown that the caudate nucleus is reduced in size in alcoholic subjects.

This latter study highlights the importance of a multidisciplinary approach to alcohol-related brain damage, each discipline providing new data and ideas that give direction for further research. The use of new techniques for the objective evaluation of each brain region is the key to progress. Many workers have failed to identify abnormalities because of technical limitations in their approach. A combination of loss of nerve cells and shrinkage of neuropil can result in normal nerve cell counts per unit area or volume, and it is only when the entire anatomical region is studied that the abnormality becomes evident. This is particularly important for complex anatomical structures like the NbM, where studies have shown that particular regions (anterior and posterior poles) are more susceptible to damage (Harper et al. 1991).

Clarification of the pathogenesis of alcohol-related brain damage is occurring slowly. The three main contenders are alcohol per se or its metabolites, associated vitamin B deficiencies (especially thiamine), and changes secondary to cirrhosis of the liver. The evidence would suggest that cirrhosis is the least important. For the other two factors, there seems little

doubt that different regions of the brain are more susceptible to the effects of one or the other mechanism. The cerebellum seems more susceptible to damage because of thiamine deficiency, whereas the frontal cortical neurons are more susceptible to alcohol or its metabolites.

The key to further studies of pathogenesis lies in the pathological examination of cases that can be accurately classified as either moderate drinkers, uncomplicated alcoholics, alcoholics with proven thiamine deficiency (clinical or pathological evidence of WKS), or alcoholics with cirrhosis. Two other important groups to study are *nonalcoholic* WKS and cirrhosis cases. The ultimate goal would be to mount a study similar to that of Victor et al. (1971), in which patients had been studied in detail during life and after death. With new noninvasive tools such as CT and MRI, combined with much more sophisticated neuropsychological techniques, the results of quantitative neuropathology would have far more meaning. The same studies could well provide us with dose/time/effect relationships, although this is a particularly difficult question to address in human studies, given the long drinking histories of most patients, the variability in drinking patterns with age, sex, and time, and difficulties with data retrieval. This question is more likely to be answered using animal models, but at least we could provide rough guides as to "safe" levels of drinking that do not cause any identifiable brain damage. The Australian government currently recommends "safe" levels of less than 20 grams per day for women and 40 grams per day for men (Pols and Hawks 1986).

It should be noted that there is good clinical, radiological, and neuropathological evidence that some alcohol-related brain damage is reversible following prolonged periods of abstinence (Carlen et al. 1978; McMullen et al. 1984; Jacobson 1986). One can speculate on the structural change(s) underlying this reversible brain shrinkage. Both neurons and their myelinated axons seem involved. Neuronal dendritic networks provide an enormous surface area for reception of information and interaction with other neurons. This network changes constantly.

Human and experimental evidence indicates that retraction of this network is caused by chronic consumption of alcohol (Pentney 1982; Harper and Corbett 1990). McMullen et al. (1984) showed an expansion of the dendritic arbor following a withdrawal period from alcohol, indicating reversible brain damage. Myelinated axons form the bulk of the white matter, which is the principal region of shrinkage in alcohol-related brain damage. Experimental data showing structural changes in the myelin sheaths caused by pre- and postnatal alcohol exposure (Phillips et al. 1991) lend weight to the hypothesis that these changes in white matter could be a major factor in causing brain shrinkage. Moreover, with a significant period of abstinence, these brain alterations are potentially reversible.

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4

IN VIVO IMAGING OF MORPHOLOGICAL BRAIN ALTERATIONS ASSOCIATED WITH ALCOHOLISM

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INTRODUCTION

As documented elsewhere in this book, people with a history of heavy alcohol consumption often show cognitive and neurological impairments during life, as well as greatly reduced brain volume and characteristic lesions in subcortical brain regions post mortem. In vivo neuroimaging techniques, such as computerized tomography (CT) and magnetic resonance imaging (MRI), make it possible to quantify macroscopic structural brain changes and relate these to clinical characteristics and cognitive function while the patient is still alive. Furthermore, these techniques can be used to determine if changes progress with continued drinking or reverse during periods of abstinence. In this chapter, we will first briefly outline the brain morphology and pathology information these techniques can provide, and then describe the nature of the morphological brain changes that have been documented in chronic alcohol abusers using these techniques.

CT AND MRI MEASURES OF BRAIN MORPHOLOGY

CT images provide excellent contrast between bone, brain tissue, and cerebrospinal fluid (CSF), with bone appearing bright, CSF appearing dark, and brain tissue falling in between. Thus, the ventricles, which are filled with CSF, show up clearly on CT sections obtained in the axial plane. Because brain tissue and CSF occupy a closed space within the skull, any increase in CSF in a previously healthy brain is generally believed to result from reduced volume of the surrounding brain tissue. The numerous CT observations of enlarged ventricles in alcoholics (Lishman 1990) thus lend credence to the concept that heavy alcohol consumption leads to brain tissue loss, or brain damage. However, CT's limited capability for differentiating between white matter and gray matter precludes reliable determination of which tissue type is most affected by chronic alcohol consumption. Furthermore, beam hard-

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ening artifact on CT (see Di Chiro et al. 1978) distorts the representation of tissue and fluid material adjacent to bone, making it difficult to obtain accurate estimates of the integrity of cortical sulci, and thus the cortical regions most affected. In contrast, MR images are not distorted by bone; can provide coronal, sagittal, and axial views of the brain; and yield images with better resolution and excellent contrast between white and gray matter. An additional advantage of MR imaging is that, being free of ionizing radiation, MR scans can be repeated in longitudinal studies without exposing patients to radiation risk.

Volumetric Measurements of Brain Morphology, Tissue Type, and Neuroanatomic Structures

Many different approaches have been used to assess ventricular or sulcal size on CT or MR images. One approach is to compare images with clinical standards, use a qualitative rating scale, or rank them against one another. Another approach involves linear measures of ventricular or sulcal width at various points or tracing ventricular boundaries to calculate area on individual sections.

Computerized techniques to measure the volume of these CSF-filled spaces, rather than the area of just a single slice, have also been developed and can provide an efficient and reliable approach to image analysis. One such technique involves classifying each pixel as either CSF or tissue for CT scans (Pfefferbaum et al. 1986) or as CSF and white and gray matter for MRI scans (Lim and Pfefferbaum 1989). The number of pixels assigned to each compartment in anatomically or geometrically defined regions can then be summed mathematically to provide an estimate of the volume of CSF and gray or white matter in that region of interest (ROI). ROIs can include the entire brain, each hemisphere, specific cortical lobes or segments thereof, or subcortical regions such as the diencephalon, caudate nuclei, or lenticular nuclei.

Investigators are also exploiting the fine structural resolution in MR images to measure discrete neuroanatomic structures, such as the mammillary bodies (Charness and DeLaPaz 1987) or the hippocampus (Lim et al. 1990), by outlining them as they appear on single or contiguous sections. Thus, MRI offers investigators an opportunity to assess not only global morphological changes, but also, within the limitations of available resolution, alterations in more specific neuroanatomic structures.

White Matter Lucencies

MRI not only enables in vivo quantification of the gross morphological neuroanatomic changes associated with chronic alcohol use, but also allows assessment of qualitative changes. Abnormalities, particularly around the ventricles and in deep white matter areas, are seen as hyperintensities, or lucencies, on T2-weighted MR images (Awad et al. 1986). Regions of lucency in white matter tracts have been observed, not only in the MR brain images of normal elderly subjects, but also in those of patients with a variety of mental disorders. Attempts to

understand the pathophysiology or functional significance of these MRI observations are under way. However, recent studies assessing relationships between in vivo MRI measures and cognitive performance, as well as in vivo or ex vivo MRI measures and post mortem measures, have not yet reached a consensus (Braffman et al. 1988; Fazekas et al. 1991; Grafton et al. 1991; Kirkpatrick and Hayman 1987; Mirsen et al. 1991). Although it is widely recognized that incomplete white matter infarctions (Englund et al. 1988) affect the integrity of white matter tracts, it is not yet established whether the presence of lucencies implies that these infarctions have taken place.

Relaxation Times

For a given set of acquisition parameters, the signal intensity of an MR image is determined by the spin density and relaxation rates of the protons that generate the image. Some investigators measure relaxation times (T1 and T2) within designated ROIs as the variable of interest. T1 is an exponential time constant measuring the time taken for nuclei to return to equilibrium and realign with the magnetic field, whereas T2 describes signal loss due to interference between nuclei. (T1 and T2 refer to longitudinal and transverse relaxation times, respectively.) T1 is prolonged as the proportion of free to bound water within brain tissue increases (MacDonald et al. 1986). T2 shows similar though slightly less precise function (Bottomley et al. 1984; Fu et al. 1990). Thus, T1 and T2 reflect brain hydration status and offer an approach to assessing the contribution of changes in hydration status to the morphological brain alterations seen in alcoholism (Besson 1990; Chick et al. 1989).

Magnetic Resonance Spectroscopy

Single voxel (smallest volume of resolution) spectroscopy can detect relative brain tissue concentrations of various endogenous metabolites, especially protons and phosphorus, as well as some exogenous compounds in delimited ROIs (Dager and Steen 1992). Recent developments in spectroscopic imaging now make it possible to characterize the distribution of these metabolites throughout the brain (Spielman et al. 1992). With proton spectroscopic imaging it is possible to identify peaks representing N-acetyl aspartate (observed primarily in viable neurons), creatine (an energy metabolite), and choline (a precursor to acetylcholine and a constituent of cell membrane synthesis). However, this technique has not yet been used to assess the constituency of brain tissue in chronic alcoholics. The spectroscopic visibility of ethanol has also been demonstrated (Hanstock et al. 1990) and opens the possibility of in vivo assessments of acute uptake and distribution of ethanol in the brain (Spielman et al. in press).

GLOBAL CHANGES IN BRAIN MORPHOLOGY ASSOCIATED WITH ALCOHOLISM: ROLE OF AGE, ALCOHOL CONSUMPTION, NUTRITIONAL STATUS, GENETIC VARIABLES, AND GENDER

Increases in the size of the lateral ventricles and cortical sulci associated with

chronic alcohol use were first observed in vivo in the 1960's using air encephalography (Brewer and Perrett 1971; Haug 1968), then in the 1970's using CT (Cala et al. 1978; Fox et al. 1976), and by the late 1980's using MRI (Chick et al. 1989; Zipursky et al. 1989; see also reviews by Lishman 1990; Wilkinson 1987). These changes are similar to the changes associated with normal aging (Pfefferbaum et al. 1986; Stafford et al. 1988) and raise the methodological question of how to properly evaluate neuroanatomic changes attributable to alcoholism, independently of normal aging changes. Figure 1 illustrates CT scans from 35-year-old and 61-year-old healthy men with low alcohol-drinking habits. Figure 2 illustrates CT scans from 36-year-old and 60-year-old alcoholic men. The ventricles of the 36-year-old alcoholic in figure 2 are larger than those of the 61-year-old community control in figure 1.

The methodological issue can be particularly problematic if the duration of alcoholism in a particular sample correlates highly with chronological age (e.g., older alcoholics often have been drinking

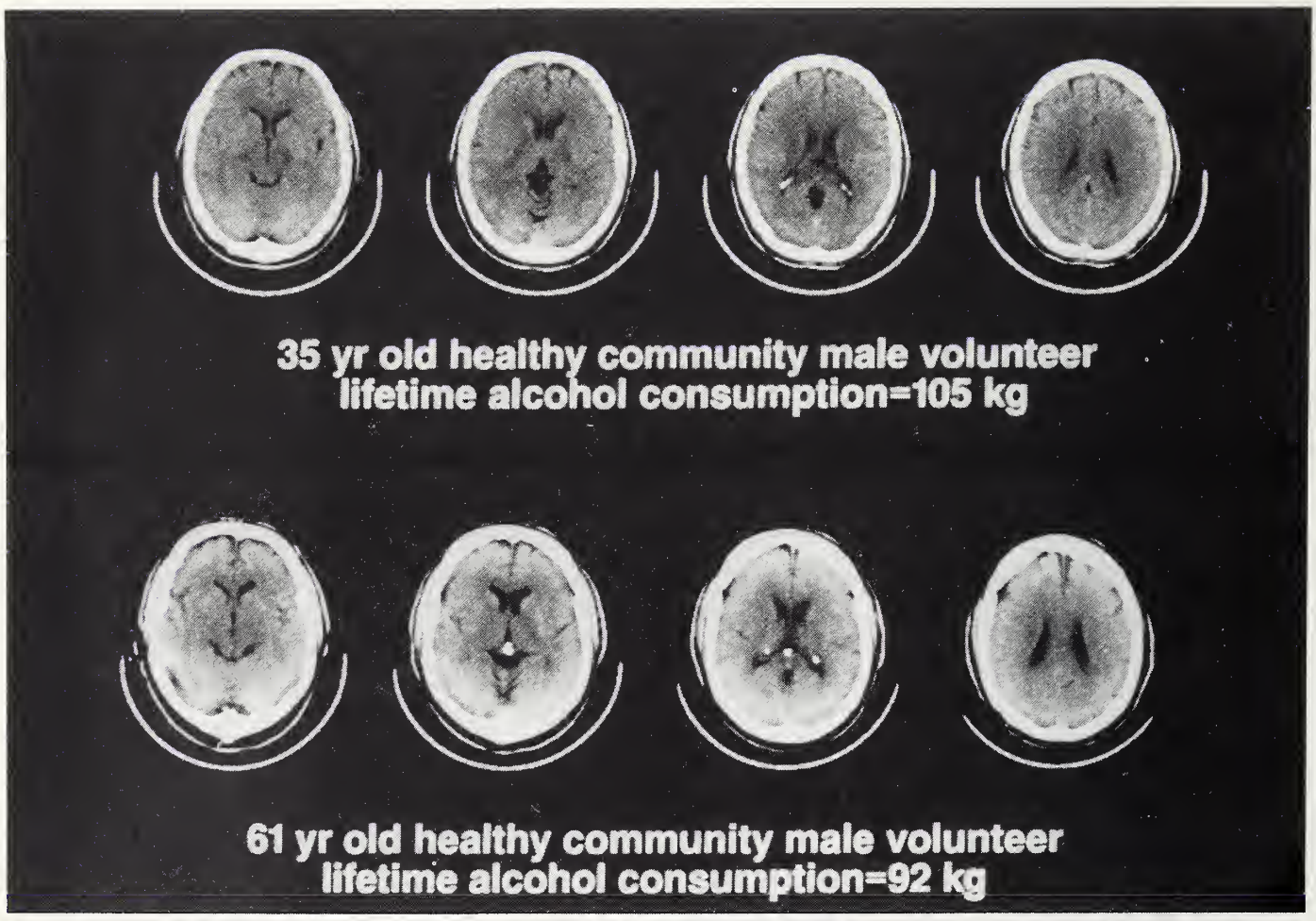

FIGURE 1

Four 10-mm thick axial CT brain sections from a 35-year-old and a 61-year-old, both healthy community volunteers who were light social drinkers. These two scans provide a cross-sectional illustration of the increase in lateral ventricles that occurs normally with aging.

for longer than younger alcoholics). Covarying for age in this situation may also exclude the influence of alcohol consumption and disease duration and thus minimize any actual associations. One approach to resolving this problem is to obtain brain morphological data from a population of healthy community controls spanning the relevant age range and use the statistical technique of regression analysis to establish age norms for each measure. Data from each alcoholic patient can then be converted into z-scores, which express the extent a patient differs from the norms for his or her age. This procedure allows one to examine the effects of disease duration and alcohol consumption while controlling for the effects of normal aging. We have implemented this approach for studying neuroanatomic variables in patients with schizophrenia (Pfefferbaum et al. 1988*b*) and Alzheimer's disease (Pfefferbaum et al. 1990). It is now proving particularly useful for evaluating brain morphological changes seen in alcoholics (Jernigan et al. 1991*b*; Pfefferbaum et al. 1992; Pfefferbaum et al. 1988*a*).

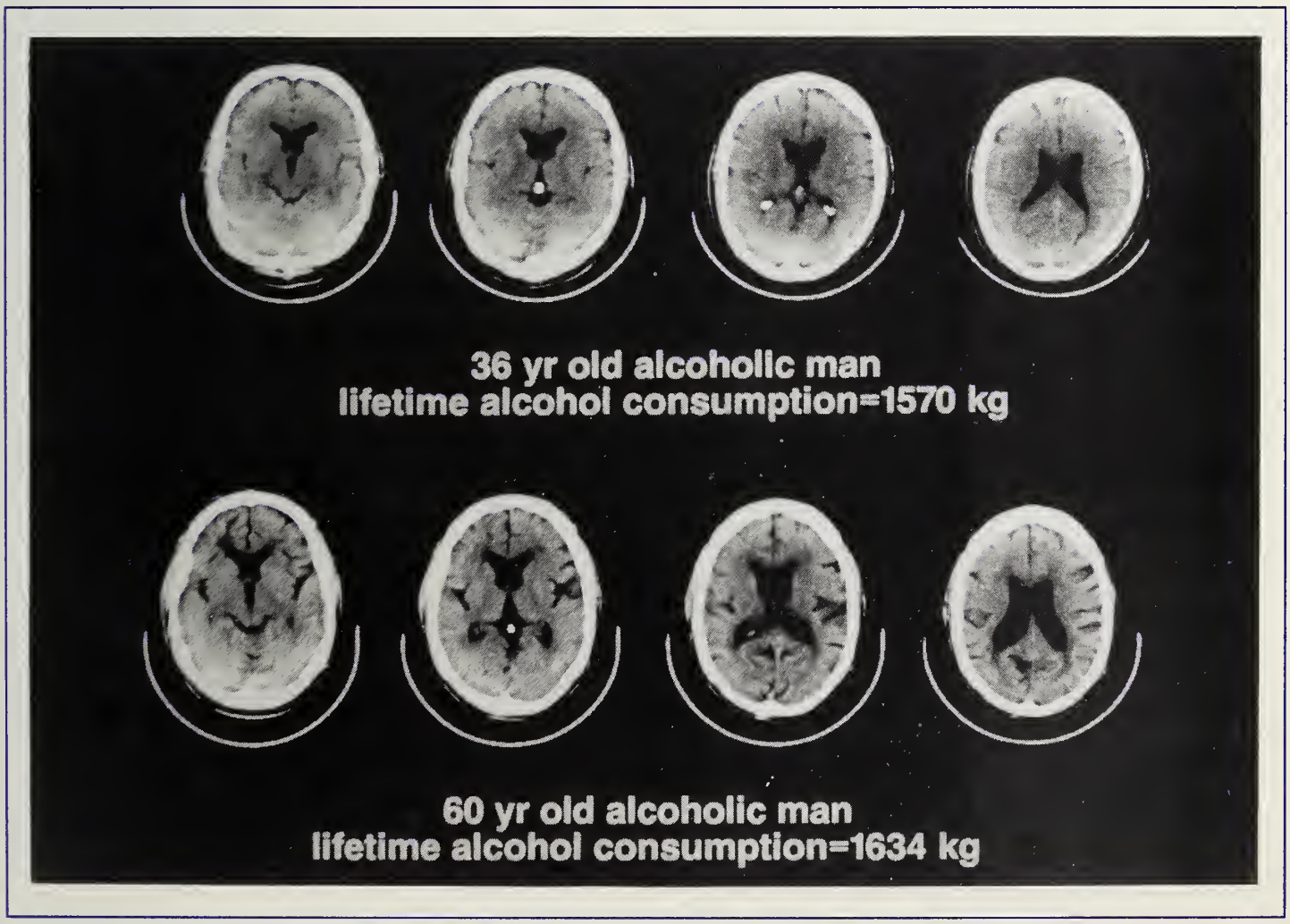

FIGURE 2

Four 10-mm thick axial CT brain sections from a 36-year-old man and a 60-year-old man, each with histories of heavy alcohol consumption. The lateral ventricles for each man are considerably larger than those seen in their age-matched control.

MRI studies have confirmed and extended CT studies by showing that with chronic alcohol abuse CSF spaces increase not only subcortically (lateral and third ventricles) but also cortically, with enlarged sulci and subarachnoid spaces affecting all cortices. Furthermore, MRI has shown that increases in CSF volumes are accompanied by reductions in gray (Jernigan et al. 1991*b*, Pfefferbaum et al. 1992) and white (Pfefferbaum et al. 1992) matter volumes, as well as an increase in areas of white matter hyperintensity (Gallucci et al. 1989; Jernigan et al. 1991*b*). The involvement of white matter confirms neuropathological studies, which have reported white matter volume reduction (de la Monte 1988; Harper and Kril 1985; Harper et al. 1985). The failure to observe at autopsy the gray matter volume changes seen in vivo may be due to artifacts in specimen preparation and fixation affecting gray matter specifically (gray matter has a higher water content than white matter). It may also be due to the failure to adequately control for the confounding effects of normal age-related changes on gray matter (Jernigan et al. 1990, 1991*a*; Lim et al. 1992; Pfefferbaum et al. 1992).

Using an age regression model, Pfefferbaum et al. (1988*a*, 1992) have found that CSF volumes exceed age norms to a greater extent in old than young alcoholics. This finding could reflect the cumulative effect of a lifetime of alcohol exposure, the greater vulnerability of the aging brain, or some combination of both. In the first (CT) study (Pfefferbaum et al. 1988*a*), 37 male veteran alcoholics, ranging in age from 26 to 62 years, were studied. In this sample, age and lifetime consumption of alcohol were related; that is, many older alcoholics had been drinking all their lives. Thus, they not only had older brains, but also had experienced the cumulative toxicity of a lifetime of alcohol exposure.

The second (MRI) study (Pfefferbaum et al. 1992) of 49 veteran alcoholics, ranging in age from 23 to 70 years, also found that CSF volumes exceed age norms to a greater extent in old than young alcoholics. In this sample, however, there was no relationship between age, disease duration, or total lifetime intake of ethanol. Nor was there any relationship between lifetime alcohol consumption and brain abnormalities. Many of the older alcoholics had relatively short drinking histories. The results of this study are consistent with the proposal that the accelerating association between age and brain damage can be attributed more to the greater vulnerability of the aged brain than to the cumulative effects of a lifetime of exposure to alcohol.

This conclusion is also consistent with other studies of the effects of alcohol in the elderly. For instance, peak blood ethanol concentration after an acute fixed dose of alcohol increases with age and may be attributed to reduction in lean body mass and total body water occurring with age (Vestal et al. 1977). Furthermore, although gastric alcohol dehydrogenase activity decreases significantly with advancing age in men (Seitz et al. 1990), central nervous system functions are more impaired by alcohol with

advancing age, even when one controls for blood alcohol level (Vogel-Sprott and Barrett 1984).

The observed reduction in brain tissue in alcohol-dependent patients is the result of a multifactorial process. A primary candidate is alcohol consumption itself. Direct investigations of the in vivo role of alcohol as a neurotoxic or demyelinating agent in humans is of necessity sparse. Animal models provide support for the notion that ethanol is destructive of both white matter (Hansen et al. 1991; Lancaster et al. 1984; Popova and Frumkina 1985; see also Lancaster, Chapter 19) and gray matter (Pentney and Quackenbush 1990). However, in humans there is often a surprising lack of association between the amount of alcohol consumed and the amount of cortical shrinkage, as measured by ventricular enlargement on CT or MRI (Lishman 1990). This phenomenon parallels the considerable range in apparent vulnerability to other physiologically devastating effects of alcohol, such as liver damage and cardiovascular disease.

Impaired nutrition is a frequent sequela of chronic alcohol abuse and one that possibly interacts with alcohol exposure to produce liver disease (Lieber 1989) and pathological changes in the brain (Pratt et al. 1990; Victor et al. 1971). Illnesses such as anorexia nervosa involving extreme malnourishment are associated with brain ventricular enlargement, which reverses to some extent with treatment (Artmann et al. 1985; Enzmann and Lane 1977; Krieg et al. 1989). However, alcoholics recruited to research protocols tend not to be severely malnourished at the time of scanning and sometimes even exceed body mass index (BMI) norms (e.g., Agartz et al. 1991), yet they still exhibit brain shrinkage. Nonetheless, in our CT study of alcoholism (Pfefferbaum et al. 1988*a*) BMI, hemoglobin, and mean corpuscular volume correlated with ventricular enlargement beyond age norms. In a more recent study (Pfefferbaum et al. 1992), BMI did not correlate with any morphological brain measure. However, age-corrected volume measures of the lateral and third ventricles and an estimate of the total intracranial CSF showed significant negative correlations with red blood count, hemoglobin, and hematocrit. Thus, it appears that anemia in these patients was associated with greater CSF and less brain tissue volume.

Considerable interest exists in the possibility of a genetic basis for susceptibility to alcohol dependence and/or vulnerability to its deleterious effects (Blum et al. 1991; Blum et al. 1990; Cloninger 1987; Schuckit 1985). However, neither twin studies nor studies that take family history of alcoholism into account in their analyses provide much support that the neuroanatomic changes described above would serve as genetic markers that precede disease onset rather than reflect its progression. Gurling et al. (1986, 1984) have studied monozygotic twins showing discordance for alcoholism and report differences in brain and ventricular volumes, as well as localized brain density measures between the severely dependent and normal drinking co-twins. Twins discordant for less severe levels of alcoholism did not show signifi-

cant differences. Ron (1983) found no difference between family-history-positive and family-history-negative alcoholics in the CT neuroanatomic measures.

Increasing interest is being paid to the phenomenon of alcoholism among women (Schmidt et al. 1990), its more intense somatic sequelae (Glenn et al. 1989; Haberman and Natarajan 1989; Jones-Saumty et al. 1981; see also Glenn, chapter 9), and mechanisms to account for these sex differences (Frezza et al. 1990). Few in vivo brain imaging studies have even reported on women, let alone systematically attempted to address the question of whether the brains of alcoholic women are more vulnerable than those of men to the effects of alcohol. This is a complex question in which sex-related physiological variables that affect metabolism such as fat/fluid body composition, body/brain size, as well as sex-related differences in patterns of alcohol consumption and recognition of alcohol dependence need careful consideration. Jacobson's CT study (1986) examined some of these issues. Its findings are consistent with studies of liver disease (Van Thiel and Gavaler 1988) and neuropsychological functioning (Hochla and Parsons 1982) in suggesting that women are more vulnerable than men to the effects of alcohol. Jacobson found that alcoholic women had similar levels of ventricular enlargement compared to alcoholic men, but after drinking less alcohol for shorter periods. These findings persisted, even after the effects of different body weight and head size were considered. Similar findings have been recently reported by Mann et al. (1992*a*). In contrast, a study of a large (n = 400) random sample of men and women from the general population (Bergman et al. 1983) found that women who were classified as "alcohol-dependent," based on their reported alcohol consumption and associated behaviors, were not significantly different in CT measures of ventricular enlargement than those classified as "low-consumers." In contrast, "alcohol-dependent" men had significantly larger ventricles than "low-consumer" men.

MRI studies of alcoholic women are underway in several laboratories, but few data have yet been published. One report, based on only 10 alcoholic women noted that all but 1 subject had ventricular volumes within normal limits for their age (Kroft et al. 1991).

LOCALIZED BRAIN DAMAGE ASSOCIATED WITH ALCOHOLISM

Any study of localized brain changes needs to be placed within the context of overall changes in gross brain morphology (Zipursky et al. 1992). Most in vivo CT or MRI studies of brain changes in chronic alcoholics have tended to emphasize generalized neuroanatomic changes inferred from increases in lateral ventricles and sulci, and overall cortical reductions in white and gray matter volumes. As discussed elsewhere in this volume (Oscar-Berman and Hutner, chapter 6), theories of the nature of brain damage associated with alcoholism, based on cognitive deficits, have suggested both frontal lobe and right hemisphere damage. While

some CT and post mortem studies have spoken in general terms of marked frontal atrophy in alcoholic patients, little systematic evidence exists to support preferential involvement of the frontal lobes. Recent MRI studies, in which the cortex was divided into ROIs designed to correspond roughly to lobar boundaries, concur that CSF increases occur throughout all peripheral cortical areas studied, rather than being confined to or even being more marked in a single region (Jernigan et al. 1991*b*; Pfefferbaum et al. 1992; Shear et al. 1992*a*).

Classically, localized brain lesions of diencephalon, seen post mortem, are considered pathognomic for Wernicke's encephalopathy and Korsakoff's syndrome—WKS as a combined taxonomy (Victor et al. 1989). Interestingly enough, such changes have also been detected, post mortem, in a few chronic alcoholics for whom WKS was not suspected during life (Harper 1983; Lindboe and Loberg 1988; Torvik 1987). In vivo observations of these subcortical regions using CT have been limited to clinical assessments. Poor resolution and the influence of bony artifacts have precluded reliable quantification of basal brain structures. Cerebellar atrophy, especially of the vermis, is also common in WKS. Investigators have noted cerebellar atrophy on CT, usually based on the presence of sulci in the cerebellar hemispheres and cerebellar vermis and the size of cisterns surrounding the brain stem in alcoholic patients (Cala et al. 1978; Carlen et al. 1986; Haubek and Lee 1979). Usually, these observations did not correspond with clinical signs of cerebellar ataxia. MRI now enables high resolution quantification of cerebellar structures (Press et al. 1990) and can help to answer questions raised by the early CT observations of clinically silent apparent cerebellar lesions in non-KS alcoholic patients. Using qualitative assessment of MRI, Davila et al. (1992) have demonstrated cerebellar hemisphere and vermal atrophy in chronic alcoholics.

Investigations of diencephalic structures have also provided hints that lesions previously considered pathognomic for WKS may also occur in alcoholic patients for whom WKS is not clinically suspected. Third ventricle width or volume has been the most commonly reported measure, as CSF increases here imply tissue loss in surrounding diencephalic structures. Some CT (Acker et al. 1987; Kato et al. 1991; Muuronen et al. 1989) and MRI (Pfefferbaum et al. 1992) studies have found enlargement of the third ventricle of chronic alcoholics without WKS. Others have only found third ventricular enlargement in patients with WKS (Jacobson and Lishman 1990; Shimamura et al. 1988). The delineation of specific diencephalic structures such as the mammillary bodies can now be accomplished using MRI, which has shown that the mammillary bodies are drastically reduced in size in patients with WKS (Charness and DeLaPaz 1987; Squire et al. 1990). However, Davila et al. (1992), using qualitative assessment of MRI, have also demonstrated mammillary body size reduction in non-WKS chronic alcoholics. Jernigan et al. have shown overall reductions in the volume of subcortical gray

matter in both WKS and non-WKS chronic alcohol abusers (Jernigan et al. 1991*b*,*c*). However, their anterior diencephalic measure, which encompassed anterior hypothalamic and septal structures, was significantly reduced in WKS patients but not in non-WKS alcoholics. Their measure of the caudate nucleus was reduced in both groups.

Observations of subcortical white matter abnormalities in MRI studies of relatively unimpaired alcoholics (Gallucci et al. 1989; Jernigan et al. 1991*b*; Pfefferbaum et al. 1992) are also consistent with the pathological observations that focal brain damage as well as global shrinkage is present in most alcoholics who do not have obvious WKS (see Harper and Kril, chapter 3).

REVERSIBILITY AND PROGRESSION OF BRAIN DAMAGE ASSOCIATED WITH ALCOHOLISM

The possibility that abstinence could reverse alcohol-related neuroanatomic brain changes was first demonstrated by Carlen et al. (1978) using CT. Subsequent reports of larger samples from his (Carlen et al. 1984) and other laboratories (Artmann et al. 1981; Muuronen et al. 1989; Ron 1983) have confirmed reversibility of ventricular enlargement in a proportion of abstinent alcoholics over periods of months to years. Muuronen et al. (1989) performed a 5-year followup of a sample of alcoholic patients initially scanned in 1977–79. Of the 37 patients retested, 16 were classified as abstinent and 21 were still drinking. Ventricular size at followup was reduced relative to the first assessment in the abstainers but not in the drinkers, implying some reversibility. Ventricles in the abstainers at followup, however, were still enlarged relative to a nonalcoholic control group. Work in progress at the San Diego DVA Medical Center following alcoholics for 3 months after completion of a treatment program has found reduction in overall CSF volume in abstainers but not in those who resumed drinking. CSF reductions were associated with white matter increases in the abstainers, suggesting that remyelination may play a role in the reversibility effect (Shear et al. 1992*b*).

Further studies of the time course of reversibility and mechanisms underlying this process are currently under active investigation. The importance of demonstrating test-retest reliability of brain image measures cannot be overstated, if this phenomenon is to be adequately documented and understood. Changes in the positioning of subjects in the scanner on a followup scan can introduce error of the same degree of magnitude as the effect under observation. Replacement of one generation scanner by the next introduces additional "noise" into the measurement of change. The reversibility effect is quite small and requires reliable quantitative techniques to be detected. In one study (Zipursky et al. 1989), ventricular volume declined approximately 15 percent during withdrawal. In one of the reports of Carlen et al. (1984), long-term reversibility was greater in patients who had their first scan shortly after their last drink, implying that reversibility was mainly accomplished during the initial withdraw-

al period. Some studies, using MRI (Schroth et al. 1988; Zipursky et al. 1989), have since established that some reversibility does occur within the first few weeks of abstinence. However, one study testing at 36 hours and 10 days after last drink found no change in CT measures (Claus et al. 1987).

The fact that some reduction in brain tissue and increase in CSF volume may be reversible has led to the use of the term "shrinkage" rather than "atrophy" because the underlying process is not clear. One hypothesis regarding the reversibility of ventricular and sulcal enlargement with abstinence is that the apparent increase in brain tissue and decrease in CSF with abstinence is due to relative brain tissue rehydration during withdrawal. An approach to investigating this hypothesis is to measure the longitudinal relaxation time (T1) of protons from magnetic resonance images of the brain. T1 is prolonged as the proportion of free to bound water within brain tissue increases (MacDonald et al. 1986). T2 shows a similar though slightly less precise function (Bottomley et al. 1984; Fu et al. 1990). CSF has longer T1 and T2 than tissue, and gray matter has a higher water content and thus a longer T1 than white matter.

Despite some elegant findings with animal models and the apparent face validity of the relaxation times as a measure of brain hydration, application of this technology to the study of alcohol withdrawal has produced inconsistent results. Since 1981, when Besson et al. reported shorter brain T1s in both gray and white matter in intoxicated alcoholics, which lengthened during withdrawal and abstinence (1 to 6 weeks), there has been a series of studies, many with limited control data, that sampled at different time points and reported conflicting results (Besson et al. 1981, 1989*a*; Schroth et al. 1988; Smith et al. 1985, 1988). Chick et al. (1989) found longer T1s in gray and white matter in the alcoholics measured 2 weeks postwithdrawal, whereas Agartz et al. (1991), in a study of comparable design, but using a lower field instrument, found no differences in T1 or T2 between alcoholics and controls. Both studies found correlations between atrophy and T1 (Mander et al. 1989), suggesting that partial voluming (some CSF present in what appears to be a tissue voxel) may contribute to the increased T1 seen in alcoholics. A recent report from Mann et al. (1992*b*) on a 5-week followup of abstinent alcoholics reports a significant drop in CSF volume, but no increase in T2 times, which would have been predicted if brain tissue rehydration underlay CSF reduction seen with abstinence.

In an autopsy study, Harper et al. (1988) concluded that brain shrinkage in alcoholics is not caused by changes in hydration. Drinking status of their sample at time of death, which may have a profound bearing on the state of hydration of the alcoholics' brains, was not reported. An interesting animal experiment (Besson et al. 1989*b*) demonstrated increased brain T1 in rats fed alcohol for 6 months, but only at 1 month after withdrawal. Furthermore, the authors claimed that there were no changes in T2 or water content. They concluded that the T1 effects represent alterations in the

cell membrane instead of changes in hydration.

In summary, the data on T1 and its relationship to brain hydration are conflicting. Besides possible unreliability of this measure (Bottomley et al. 1987), crucial factors appear to be the time following ethanol withdrawal, adequate tissue sampling to avoid partial voluming effects, and the need for nonalcoholic control values.

CONCLUSION

Neuroimaging studies have demonstrated that chronic alcoholism produces widespread brain shrinkage, especially in older individuals. Some of this shrinkage may be reversible with abstinence, but the mechanism is yet to be elucidated. There may well be regionally specific tissue damage in addition to the generalized shrinkage, which could be of considerable clinical import. MRI studies are now beginning to examine the effect of excessive alcohol consumption on many specific brain ROIs. Much remains to be examined in terms of the prognostic significance of the observed brain morphologic sequelae of alcoholism as well as the role of these changes in cognitive function. MRI especially offers a unique opportunity to observe brain morphology in living alcoholics as the disease progresses or remits. Developments in MR spectroscopic imaging and studies of proton relaxation times have promise for adding metabolic and physiologic data to MR structural information.

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5

NEUROPHYSIOLOGICAL FACTORS ASSOCIATED WITH ALCOHOLISM

Bernice Porjesz and Henri Begleiter[1]

INTRODUCTION

Event-related potential (ERP) techniques permit the observation of electrophysiological manifestations of cognitive activity and thereby offer a unique approach for assessing brain function. ERPs are obtained by recording, with noninvasive scalp electrodes, the time-locked brain electrical activity following the delivery of a discrete stimulus to any sensory modality. Signal-averaging techniques permit the extraction of these time-locked neuroelectric signals from the random electroencephalogram (EEG) background "noise," which is canceled out with these procedures. Depending on stimulation properties, experimental paradigms, filter settings, recording sites, feature extraction, and quantitative measurement procedures, these time-locked signals represent overlapping activity emanating from neural generators along the pathways from peripheral end organs to higher cortical integrative centers in the brain. Thus, the functional integrity of many systems in the brain can be assessed.

ERP techniques are also useful in indexing electrophysiological concomitants of complex cognitive tasks (Hillyard et al. 1978; Donchin 1979; Donchin et al. 1978). ERPs consist of characteristic, highly reproducible waveforms. The early components of the waveforms, occurring less than 100 milliseconds after stimulus presentation, reflect stimulus characteristics (e.g., intensity). Because such components are considered to be determined by physical properties of the stimuli, they customarily are referred to as evoked potentials (EPs). In contrast, the later ERP components are influenced more by psychological factors such as the salience of the event.[2]

EPs are extremely sensitive to the various aspects of acute and chronic alcohol administration on the brain, specifically alcoholization, tolerance, withdrawal, and long-term brain effects. Alcoholization is characterized by decreases in EP

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[2]*For a detailed discussion of measurement and application issues of the ERP, the interested reader might consult Begleiter (1979) or Coles et al. (1986).*

amplitudes (Bierley et al. 1980), particularly the negative component (N1) occurring 100 milliseconds after the stimulus (Porjesz and Begleiter 1992), as well as decreases in conduction velocities of both the brain stem auditory evoked response (BAER) (Squires et al. 1978*a*,*b*; Chu et al. 1978) and the positive component occurring at 300 milliseconds (P3) (Schuckit et al. 1988; Porjesz and Begleiter 1992). When tolerance develops, the BAER delays are less pronounced (Squires et al. 1978*a*,*b*; Chu et al. 1978; Zilm et al. 1981), and P3 latency recovers relatively quickly (Schuckit et al. 1988; Porjesz and Begleiter 1992).

Withdrawal is marked by increases in EP voltages and extremely shortened BAER latencies, suggesting underlying central nervous system (CNS) hyperexcitability (Porjesz et al. 1976; Begleiter and Porjesz 1977, 1979; Begleiter et al. 1980*a*; Squires et al. 1978*a*,*b*; Chu et al. 1978; Hunter and Walker 1980; Romani and Cosi 1989; Noldy and Carlen 1990; Neiman et al. 1991). Long-term abstinence following chronic alcohol intake is characterized by depressed EP amplitudes (hyporeactivity) and prolonged BAER latencies and slower conduction velocities (Begleiter et al. 1981; Porjesz and Begleiter 1983, 1985). This chapter provides an overview of the changes associated with alcohol in ERPs and EPs.

SENSORY EVOKED POTENTIALS

BAERs

The BAER provides sensitive measures of subcortical functioning along the auditory pathway with a single noninvasive scalp electrode (Jewett and Williston 1971; Sohmer and Feinmesser 1967). These "far-field" potentials consist of seven time-locked positive peaks. Each peak is presumed to reflect activity at sites along the auditory pathway from the auditory nerve through the brain stem (Buchwald and Huang 1975; Jewett 1970; Lev and Sohmer 1972; Starr and Achor 1975; Starr and Hamilton 1976; Stockard and Rossiter 1977). The latencies of these peaks, as well as their central conduction velocities (time interval between the various sites), are informative in localizing pathology from the eighth nerve to the brain stem. The time interval between peak I and peak V is taken as the measure of brain stem transmission time (Fabiani et al. 1979).

We found that hospitalized alcoholics abstinent for 1 month, without overt signs of neurological damage, manifest delays in latencies and brain stem transmission times of peaks II to V (Begleiter et al. 1981). These results have been replicated in neurologically intact alcoholics (Cassavan et al. 1984), and similar findings have been reported in neurologically impaired alcoholics (Chu and Squires 1980; Chu et al. 1982; Chu and Yang 1987; Haas and Nickel 1981; Nickel and Ludewig 1981; Rosenhamer and Silfverskiold 1980)

The increased transmission time has been postulated to reflect demyelination, a process that has been long suspected in alcoholics (Adams et al. 1959; also see Harper and Kril, chapter 3) and has been observed in rats chronically exposed to alcohol (Moscatelli and Demediuk 1980; also see Lancaster, chapter 19). Although a number of nutritional factors and drink-

ing history are suspected to result in BAER delays, (e.g., years of alcohol abuse, amount consumed per occasion, number and severity of withdrawals), the etiology of abnormal BAERs in alcoholics remains undetermined. There is abundant evidence that nutritional deficits lead to demyelinating diseases such as polyneuropathy (Hillman 1974) and may be necessary for BAER abnormalities (Chu et al. 1978). Overall, these findings indicate that BAER aberrations in alcoholics may be the result of alcohol and/or nutritional factors.

P1

Another useful technique in the early diagnosis of demyelinating disorders in the visual system is the pattern-reversal evoked potential (PREP) technique. This technique consists of the rapid alternation of checkerboard patterns with illuminated and nonilluminated squares. The PREP includes a prominent, positive component around 100 milliseconds (P1) poststimulus change. It is sensitive to changes in the integrity of the visual system (Halliday 1978; Halliday et al. 1973*a*,*b*; Regan et al. 1976) and is useful as an early diagnostic tool of neurological disorders such as multiple sclerosis, optic neuritis, and compression of the optic nerve (Halliday et al. 1973*a*,*b*; Hennerici et al. 1977). Abnormal delays in the P1 have been reported in several laboratories in at least 50 percent of alcoholics (Janaky et al. 1980; Porjesz and Begleiter 1983; Posthuma and Visser 1982)

In summary, these early sensory potentials (BAER and P1) are sensitive to alcohol-related aberrations. Delayed latencies in these early potentials are suggestive of possible demyelination of both auditory and visual pathways. For additional information on alcohol-related damage to cerebral white matter, consult Lancaster (chapter 19).

EVENT-RELATED POTENTIALS (ERPS) AND COGNITIVE PROCESSES

ERP techniques have proven useful in indexing electrophysiological concomitants of complex cognitive tasks (Donchin 1979; Donchin et al. 1978; Hillyard et al. 1978). They can be recorded in conjunction with or without overt behavioral responses to both attended and unattended stimuli. In contrast to other imaging techniques, ERPs reflect subtle dynamic millisecond to millisecond transactions that are elicited while the brain is being challenged. Therefore, they are more sensitive to specific brain processes than magnetic resonance imaging (MRI) or computed tomography (CT), both of which typically measure static gross brain damage. ERP abnormalities are often observed in the absence of brain damage as visualized on CT or MRI.

N1 (Nd)

The N1 component is a negative component occurring approximately 100 milliseconds after a stimulus. In healthy individuals, it is larger in response to stimuli in a relevant (or attended) channel and reduced in response to stimuli in an irrelevant channel. The Nd (or negative difference) component is a subtraction between the N1 amplitudes in the waveforms obtained to the attended and unat-

tended channels. Hence, Nd amplitude indexes the selection of a relevant channel and is related to allocation of attentional resources (Hillyard et al. 1973, 1978; Picton and Hillyard 1974).

We examined the ability of abstinent alcoholics to focus on a relevant stimulus modality and inhibit responding to an irrelevant modality by studying the N1 component of the ERP (Porjesz and Begleiter 1979). Alcoholics were presented with sequences of randomized single flashes and single clicks interspersed with rare double flashes and double clicks. For each sequence, they were instructed to count either the double flashes or double clicks, or to ignore all stimuli in otherwise identical stimulus sequences. ERPs were recorded only to the irrelevant single flashes, which were either in the relevant stimulus modality (when double flashes were counted) or irrelevant stimulus modality (when double clicks were counted), depending on the assigned condition. These frequent flashes elicited N1 components that were differentially enhanced in response to stimuli in the relevant channel (stimulus modality). That is, N1 was larger in response to the single flashes if subjects were counting the double flashes as opposed to counting the double clicks.

Using this paradigm, alcoholics demonstrated essentially normal early components (< 100 milliseconds) but significantly reduced late components. As expected, control subjects produced significantly enhanced N1 components in response to stimuli in the relevant as compared to the irrelevant modality. Alcoholics, on the other hand, maintained the same low N1 amplitudes (i.e., showed reduced Nd) regardless of the degree of task relevance. These findings suggest that alcoholics may be incapable of appropriate sensory filtering, being unable to neurophysiologically differentiate between relevant and irrelevant channels. (See Nixon, chapter 10, for further discussion of relevance/irrelevance and efficiency in alcoholics).

Using a similar bimodal experimental paradigm, Patterson et al. (1987) also examined N1 amplitudes to visual and auditory stimuli in abstinent alcoholics. Because these results were modality specific, Patterson et al. attributed the observed pattern to a sensory deficit in alcoholics in the visual but not the auditory modality. The alcoholics in their study also showed less differential enhancement in attended versus unattended visual stimuli than did nonalcoholics. However, this difference failed to achieve statistical significance. This pattern was not observed for auditory stimuli.

In an entirely visual target-selection paradigm involving geometric shapes (see section on P3 for a description), alcoholics were also found to exhibit reduced N1 amplitudes compared to controls (Porjesz et al. 1980). Despite the fact that all the stimuli in this paradigm were in the relevant channel, N1 amplitudes were found to be comparable to voltages expected in an irrelevant modality.

As suggested by the findings of Patterson et al. (1987) discussed earlier, the outcome is quite different in the auditory modality. Similarly, no differences in N1 amplitude between alcoholics and controls were reported by Pfefferbaum et

al. (1991) in both automatic and attended auditory paradigms to the frequent tones or to the rare tones in the attended paradigm. Likewise, Hertz et al. (in press) failed to obtain significant N1 differences in an auditory selective attention task.

Thus, N1 amplitude deficits are apparent in alcoholics only to visual stimuli in visual or bimodal selective attention paradigms. These visual N1 amplitude reductions are obtained in response to both the frequent nontargets and rare targets in the task-relevant channel. These results indicate that alcoholics manifest an impaired ability to selectively attend to a task-relevant sensory channel. These findings suggest that sensory-filtering mechanisms are impaired in alcoholics to visual but not auditory stimuli. Although it appears to be a modality-specific finding, differential task difficulty between auditory and visual selective attention tasks may also contribute to the observed differences.

P3 (P3a, P3b)

Considerable attention has focused on the P3 component of the ERP. The P3 is a prominent positive component occurring between 300 and 500 milliseconds after the stimulus is presented. It is elicited under rather specific conditions related to stimulus significance, namely task relevance (Sutton et al. 1967), unpredictability (Donchin et al. 1978), infrequency (Tueting et al. 1971), and certain motivational factors (Begleiter et al. 1983). P3 characteristics are unrelated to stimulus parameters and can be elicited in the absence of an expected stimulus (emitted potentials) (Klinke et al. 1968).

Two kinds of P3 are often distinguished: P3a and P3b. Most studies address the P3b component, which occurs in response to task-relevant stimuli within the subject's awareness. P3b has a parietal maximum scalp topography (Ritter et al. 1968; Simson et al. 1977*a*,*b*). In contrast, P3a is obtained in response to rare, deviant, or novel stimuli within a repetitive stimulus train, to which the subject does not attend, and has a more anterior distribution over the scalp. The most standard paradigm used to elicit a P3 is the "oddball" or target-selection paradigm. In this paradigm, subjects are asked to attend to rare target stimuli (press a button or count) while ignoring the other stimuli. ERPs in response to frequently occurring (nontarget) stimuli elicit N1 components but no P3s. Rarely occurring stimuli (targets) elicit both N1s and P3s.

In an early study mentioned previously (Porjesz et al. 1980), we studied the P3 component in abstinent alcoholics with a visual paradigm involving geometric shapes. Rare and frequent geometric shapes (e.g., triangle, square) and rare novel irregular shapes were interspersed in a random sequence. Subjects were instructed to press a button in response to the occurrence of the rare geometric shape only. Target and nontarget stimuli were alternated in blocks enabling the recording of ERPs in response to the same shape (e.g., triangle) when it was both a target and nontarget.

Using this task, alcoholics exhibited reduced or absent P3 components in response to the target stimuli without

latency delays. This finding was most pronounced over the parietal areas where the P3b is maximal (Ritter et al. 1968; Simson et al. 1977*a*,*b*). As one might expect, controls manifested differentially enhanced late P3 components in response to target stimuli. However, alcoholics produced identical low amplitude P3 waves with the same P3 latencies, regardless of whether the stimulus was a target or nontarget. Thus, the major ERP aberration shown by alcoholics was the lack of differentiation between their responses to relevant and irrelevant inputs, and the low voltages of their event-related activity. The finding of reduced P3 amplitudes in alcoholics in visual oddball paradigms has been replicated in several laboratories (Emmerson et al. 1987; Patterson et al. 1987; Pfefferbaum et al. 1991; Porjesz et al. 1987*a*). This pattern suggests underlying brain dysfunction that impairs sensory-filtering and probability-matching processes.

However, to clarify the role that difficulty of discrimination might play in determining the amplitude of P3, another visual oddball task was used (Porjesz et al. 1987*a*). P3 components were obtained in response to two targets: an easily discriminated target line stimulus that was 90 degrees from the vertical nontarget and a target that was difficult to discriminate, being only 3 degrees from the nontarget. Consistent with previous work, alcoholics produced significantly decreased P3 amplitudes. This diminished amplitude was more apparent for the easy (90-degree) target than the difficult (3-degree) target, with controls manifesting extremely large voltages in response to the easy targets. Furthermore, the amplitude difference between the easy and difficult targets was significant for controls but not for alcoholics.

Among controls, enhanced P3 in response to the easy target would be predicted based on a number of studies demonstrating that the more deviant a rare stimulus is from the background (i.e., the more easily discriminable it is), the larger the P3 amplitude (Ford et al. 1979; Johnson and Donchin 1978; Ritter et al. 1972; Ruchkin and Sutton 1978; Towey et al. 1980). Perhaps the lack of a P3 amplitude difference between the easy and difficult targets in the alcoholic group reflects an uncertainty of the correctness of their response.

Using a visual oddball task, Emmerson et al. (1987) found that only the amplitude of the N2-P3 peak-to-peak measure differentiated alcoholics from nonalcoholics. However, in order to control for a number of subject variables, they examined only alcoholics who were younger than 40 and who had been abstinent at least 1 month. Other researchers have also reported decreased P3 amplitudes without latency delays (Patterson et al. 1987; Pfefferbaum et al. 1991).

In addition to standard oddball paradigms, other visual P3 tasks also elicit diminished P3 amplitudes in alcoholics. Using a visual paradigm requiring subjects to respond on some trials (Go), but not on others (No-Go), Pfefferbaum et al. (1987) obtained lower P3 amplitudes in alcoholics under the Go but not under the No-Go conditions. In addition, the scalp distribution of P3 was more similar

across electrodes for alcoholics, compared to the controls in the Go but not in the No-Go condition.

In a visual P3 paradigm involving incentive factors, Porjesz et al. (1987*b*) reported lower P3 amplitudes to equiprobable high-incentive stimuli in alcoholics when compared to controls. No differences in latency were observed. Latency corrected average procedures indicated these results were not due to latency jitter in the average but rather were due to lower single trial voltages. This result was recently replicated by Pfefferbaum et al. (1991) in both visual and auditory oddball paradigms.

The relation between visual P3 amplitude (in the geometric shape paradigm described above) and structural brain damage as assessed by CT scans was investigated by Begleiter et al. (1980*b*). Two groups of alcoholics were studied: those who exhibited severely widened cortical sulci (Pos-CT) and those who did not manifest such widening (Neg-CT). The two groups did not differ in terms of age, education, and duration or amount of alcohol consumed. Alcoholics in the Pos-CT group showed significantly lower P3 amplitudes to target stimuli than did Neg-CT subjects. Both groups displayed significantly smaller P3 amplitudes to targets than did control subjects. Neocortical shrinkage alone cannot explain the results of diminished P3 amplitudes in alcoholics because both Pos-CT and Neg-CT alcoholics manifested these electrophysiological deficits.

Evidence from intracranial recordings in humans implicates both the medial temporal lobe (Halgren et al. 1980; McCarthy 1985; Smith et al. 1986; Stapleton 1985; Wood et al. 1980, 1984) and source(s) within the frontal lobe (McCarthy 1985) as contributing to P3 generation. These findings coupled with the rather small effect of unilateral temporal lobectomy on scalp P3 during auditory discrimination tasks (Stapleton 1985; Wood et al. 1984) suggest that multiple brain sites may be involved. Given the data suggesting frontal lobe involvement in alcohol-related cognitive deficits, it may be that the reduced P3 amplitudes in alcoholics reflect frontal lobe damage (also see Oscar-Berman and Hutner, chapter 6).

The P3 results from auditory paradigms are not as consistent as those from visual paradigms. In an early auditory oddball study in which speed of response was stressed, Pfefferbaum et al. (1979) reported no difference in P3 amplitudes between an older sample of alcoholics and healthy controls. However, alcoholics did exhibit delayed P3 latencies. In contrast, Patterson et al. (1987) found decreased auditory and visual P3 amplitudes to target stimuli in the absence of latency delays in a bimodal study. In a subsequent study (Parsons et al. 1990), this team failed to replicate the finding with female alcoholics.

In a more recent study, Pfefferbaum et al. (1991) reported that P3 amplitudes to attended target stimuli were significantly different between alcoholics and controls. This difference was found in both visual and auditory oddball paradigms. Consistent with the study by Porjesz et al. (1987*b*), single-trial latency adjustment

procedures indicated that these amplitude differences were due primarily to signal size differences between the two groups rather than greater single-trial latency variability. Single-trial analysis of P3 amplitude indicated that the reductions in amplitude were due to smaller voltages on individual trials, not the number of P3s in the average. Furthermore, standard deviations of single-trial P3 amplitude indicated no significant differences between groups for either the auditory or visual paradigm. P3 latencies were delayed in alcoholics only to attended auditory targets. There were no P3 delays to attended visual targets or unattended rare auditory stimuli.

The study by Pfefferbaum et al. (1991) clarifies earlier differences in the literature regarding whether alcoholics manifest P3 latency delays in oddball tasks. Latency delays were reported in an auditory oddball paradigm (Pfefferbaum et al. 1979) but not in a visual paradigm (Pfefferbaum et al. 1987; Porjesz et al. 1980). Similarly, Pfefferbaum et al. (1991) reported response time (RT) delays in alcoholics to auditory but not visual attended targets. Finally, Porjesz et al. (1987*a*) found P3 latency delays in alcoholics in an easy, but not a difficult, discrimination task.

Perhaps, rather than being modality specific, P3 latency delays in alcoholics are sensitive to the difficulty of discrimination between targets and nontargets. Auditory oddball tasks are generally easier than visual tasks. In the Porjesz et al. (1987*a*) study, controls demonstrated significantly earlier P3 latencies to easy discriminations as compared to difficult ones. However, alcoholics did not manifest differences in P3 latency contingent on discrimination difficulty. In alcoholics, the P3 latency delays in response to the easy targets were prolonged, comparable to latencies found with a difficult discrimination task. These results suggest that alcoholics found both tasks difficult and adopted an undifferentiated mode of responding regardless of task requirements.

Considering this literature, the most consistent electrophysiological measure that differentiates alcoholics from controls is their decreased P3 amplitude to targets for visual tasks (particularly at the parietal-midline electrode). Although these findings have also been observed in the auditory modality, they are not robust. It should be noted that evidence indicates that the visual and auditory P3s are generated at different brain loci. Furthermore, many different kinds of P3 are elicited under various experimental conditions with different brain generators (Ruchkin et al. 1990). The P3 components discussed thus far are obtained to attended stimuli of significance (i.e., to which the subject must make a response, P3b). However, a small number of studies have examined automatic processes and the more frontal P3 component associated with such processes, the P3a.

In an inattentive auditory oddball paradigm, Pfefferbaum et al. (1991) found that although P3a amplitudes in response to rare unattended stimuli were smaller in alcoholics than in controls, this result did not reach significance. However, this study used a somewhat small sample size

(23 alcoholics and 21 controls). Perhaps with a larger sample, the difference would have attained statistical significance.

Using an almost identical automatic auditory oddball paradigm in our laboratory, Realmuto et al. (in press) found that alcoholics exhibited significantly lower P3 amplitudes than did controls to rare unattended tones. In this study, 63 male alcoholics were compared to 27 controls. In addition to differences in sample size, another possible difference between the two studies is that our laboratory used tones of two different frequencies, whereas Pfefferbaum et al. (1991) used a white tone burst as the rare stimulus. Therefore, the two studies may have differed in terms of stimulus deviance of the rare stimulus relative to the background stimuli.

Both studies agree that there are no significant differences in midline topography (i.e., distribution across frontal, central or parietal midline sites) between the two groups in terms of the P3a amplitude to unattended rare stimuli. Furthermore, although Pfefferbaum's results were not significant, the direction of the difference was consistent with the findings of Realmuto et al. (in press).

Using a selective attention auditory task, Hertz et al. (in press) also found significantly lower P3 amplitudes for both attended and unattended rare tones in alcoholic subjects compared to controls. P3 latencies were delayed in response to rare nontarget but not to rare target auditory stimuli.

Thus, these studies suggest that automatic match/mismatch processes as well as control processes are impaired in alcoholics, although automatic processes may not be as significantly compromised. Alcoholics apparently are less able to distinguish deviant stimuli from repetitive background stimuli. It is possible that either the template for comparison is not formed or retained, or that the match/mismatch processes themselves are impaired in alcoholics.

N2 (MMN)

Another component of the ERP that has been examined in alcoholics is the N2 component. N2 is a negative component that occurs approximately 200 milliseconds after the stimulus is presented. The N2 component of the ERP is modality specific, with a maximum amplitude over occipito-parietal scalp regions for the visual modality, and over central areas for the auditory modality. The latency of N2 is assumed to be an early index of stimulus evaluation time (Renault and Lesevre 1979): the easier a discrimination, the earlier the latency of the N2 (Gaillard and Lawson 1980; Ritter et al. 1979; Towey et al. 1980). The latency of N2 is superior to RT as an index of stimulus evaluation time because it is not confounded by the motor response. RT, on the other hand, is a complex measure of speed of information processing, contingent on the end product of stimulus evaluation, response selection and organization, and the motor response. Although reports in the literature suggest delayed RTs in alcoholics (Bertera and Parsons 1973; Talland 1963; Vivian et al. 1973), RT studies alone cannot ascertain which aspect(s) of this complex process are slowed in alcoholics.

To examine speed of stimulus evaluation in alcoholics, we designed a visual-spatial ERP RT paradigm in which the relation between difficulty of discrimination, N2 latency, P3 characteristics, and RT could be examined. The task, described previously under the section on P3, consisted of frequently occurring vertical lines (nontargets) and two kinds of rare targets, an easy-to-discriminate target and a difficult target (Porjesz et al. 1987*a*). Subjects were required to press a button to all nonvertical stimuli.

As expected, controls exhibited delayed N2 latencies to difficult discriminations. In contrast, N2 latency did not reflect discrimination difficulty in alcoholics. Alcoholics produced similar N2 latencies regardless of discrimination difficulty. Moreover, the N2 latency occurred significantly later in the alcoholics than in the controls for both the easy and difficult discriminations. These data suggest that alcoholics found both discriminations difficult and required more time for stimulus evaluation. Interestingly, the latency difference between groups was more apparent for the easy discrimination than for the difficult discrimination. These results imply that alcoholics need disproportionately more time to make an easy discrimination than to make a difficult discrimination when compared to controls.

Consistent with previous work (Naatanen et al. 1980), the amplitude of the N2 was related to degree of stimulus deviance for controls, being larger for easy as compared to difficult discriminations. However, for alcoholics, the N2 amplitude was unaffected by discrimination difficulty.

There were no significant differences in RT between the groups although alcoholics did tend to have shorter RTs. Alcoholics also produced more errors, both in terms of false alarms and misses. However, the group differences on these measures also failed to achieve significance. This response pattern suggests an emphasis on speed as opposed to accuracy (Kutas et al. 1977) and implies that alcoholics adopted a different response strategy than controls. These data also suggest a lack of inhibition in alcoholics as reflected by their apparent inability to withhold responding until the certainty of accuracy or correctness has been established.

Using an auditory oddball paradigm in which subjects attend to one set of stimuli (e.g., tones in one ear) but not another (e.g., tones in the other ear), Realmuto et al. (in press) found that controls exhibited larger N2 amplitudes in response to the rare relevant stimuli than did alcoholics at frontal and central midline sites, but not at the parietal midline site. It is noteworthy that, in these auditory paradigms, an N2 component is also obtained in reponse to unattended rare stimuli; Naatanen et al. (1980) named this negativity to unattended deviant stimuli the mismatch negativity (MMN). Realmuto et al. (in press) found that MMN amplitude was significantly reduced in alcoholics. These investigators also found a delayed latency for alcoholics that approached significance when age was parceled out (MMN latency was found to be directly related to age).

Latency delays of the N2 component have been reported in alcoholics compared to controls in a visual oddball paradigm in which only young alcoholics (<40 years of age) were accepted for study (Emmerson et al. 1987). Hertz et al. (in press), using an auditory paradigm requiring subjects to attend to a rare tone in one ear, also found that the latency of N2 was prolonged in alcoholics. In this latter study, N2 delays were obtained for both attended targets and rare nontargets.

In general, alcoholics manifest prolonged N2 latencies. Assuming that N2 latency indexes discrimination difficulty, these prolongations in N2 suggest that alcoholics have more difficulty with stimulus evaluation than controls. Thus, on the basis of both the N2 and P3 ERP component characteristics, alcoholics seem to have less efficient match/mismatch processes than controls and, hence, more difficulty evaluating the potential significance of a stimulus.

N400

Another late ERP component that has received attention is the N400 component. The N400 is a late negative component with a maximum at centro-parietal scalp, occurring approximately 400 to 600 milliseconds after incongruous semantic stimuli. Moreover, the N400 varies with semantic incongruity, phonological priming or matching, and the extent of search in memory (for review, see Kutas and Van Petten 1988).

In our laboratory, we recently completed a study examining the N400 component in alcoholics (Porjesz and Begleiter in preparation *a*). The paradigm consisted of a lexical decision task requiring subjects to indicate as rapidly as possible whether a letter string was or was not a word. Words preceded by semantically related words were more quickly recognized than were those preceded by unrelated words or nonwords. This semantic priming effect suggests that the semantic features of each word remain activated on subsequent trials, thereby reducing the threshold of hypothetical word recognition for words sharing some semantic features. The primed words used in this paradigm were simple antonyms (e.g., hot-cold).

In this semantic processing paradigm, the N400 component is elicited to the unprimed but not the primed words in normal subjects. Our results indicate that alcoholics respond to primed words in a fashion similar to unprimed words; that is, they exhibit N400s to primed as well as unprimed words. This impaired priming mechanism suggests possible semantic memory deficits in alcoholics. This study is the first to demonstrate semantic memory deficits in alcoholics using electrophysiological measures.

Memory Potentials

In order to examine mnemonic processes that are not semantically mediated, we used a modified delayed matching-to-sample task using stimuli that were difficult to name (Begleiter et al. in press). Pairs of visual line stimuli (S1 and S2) that were either simple (consisting of a few line elements) or complex (consisting of a greater number of elements) were

randomly presented. On half of the trials, the test stimuli (S2) were identical to S1; on the remainder, S2 was distinctly different from S1. After each presentation of S2, the subject indicated whether S2 matched S1 (choice RT). Accuracy and speed were equally emphasized. This paradigm elicits a waveform with a relatively negative peak around 170 milliseconds followed by a relatively positive peak around 240 milliseconds. These peaks are maximal over right temporal areas and are termed visual memory potentials (VMP).

Begleiter et al. (submitted) found that in both alcoholic and control subjects, RTs were significantly shorter for matching versus nonmatching stimuli for both the simple and complex stimuli. RTs were shorter for simple as opposed to complex stimuli. The results were similar for alcoholics and controls. However, alcoholics produced longer RTs than did controls in all conditions. As one might expect, based on the RTs, the latency of VMP was earlier for controls than for alcoholics. Matching stimuli were processed more quickly than nonmatching stimuli as evidenced by significantly earlier VMPs for matching stimuli.

VMPs yielded higher voltages to the nonmatching S2 compared to the matching S2 in controls. However, alcoholics did not manifest any difference on these measures between matches and nonmatches. This finding suggests that alcoholics could not differentiate stimuli previously seen from novel stimuli.

These data implicate deficits in the processes underlying matching-to-sample tasks in alcoholic subjects. Alcoholics' responses to nonmatching stimuli were aberrant in both their lower voltage and their lack of differentiation from identity (matching) responses.

Overview of ERPs

In summary, the results from studies examining the N2, P3, N400, and memory potential (VMP) components of the ERP indicate that match/mismatch processes are impaired in alcoholics. P3 studies indicate that alcoholics are not only deficient in their response to task-relevant target stimuli (P3b) but also to task-irrelevant rare stimuli (P3a). Thus, P3 deficits may be attributable to malfunctioning of more rudimentary match/mismatch processes, in which the template is either lost or absent. Coupled with those results indicating delays in N2 latency, these studies indicate that alcoholics have difficulty with stimulus evaluation. Specifically, it appears that match/mismatch processes are less efficient in alcoholics, are less well localized, and require more time to occur.

In addition to the implications of the P3 paradigms, potential memory dysfunction in alcoholics is also suggested by the results from semantic priming and matching-to-sample paradigms. Alcoholics respond to primed words in a similar fashion as to unprimed words (N400). Similarly, they do not electrophysiologically discriminate between matching and nonmatching visual stimuli (VMP).

Thus, the memory dysfunction suggested by these studies appears based on deficits in rudimentary match/mismatch processes, regardless of type of stimuli or the automaticity of the task. The deficit is

most apparent under mismatch conditions, wherein controls exhibit large differential responses. However, it should be noted that additional research designed to specifically address the relation of these electrophysiological findings to direct tests of memory function is still needed and is ongoing in our laboratory.

RECOVERY OF EVOKED BRAIN POTENTIAL DEFICITS WITH ABSTINENCE

The EP is extremely sensitive not only to alcohol administration but also to subsequent withdrawal and long-term abstinence. Because of this sensitivity, it is difficult to determine whether brain dysfunction shown by alcoholics is the direct result of their time in recovery. Earlier EP studies investigating recovery in alcoholics considered the first 3 or 4 weeks after detoxification and often overlooked the effects of medication administered during treatment (e.g., Coger et al. 1976; Salamy and Faillace 1980). In these studies, disulfiram and/or chlordiazepoxide, both of which affect EP voltages, were administered to recovering alcoholics. Increased EP amplitudes have been reported in volunteers who were experimentally administered disulfiram (Peeke et al. 1979). Therefore, it is difficult to ascertain in these early studies whether the changes in amplitude reported were due to the effects of subsiding withdrawal, medication, an interaction between detoxification and medication, or recovery from brain damage. Furthermore, the study by Coger et al. (1976) used a cross-sectional design in which different groups of alcoholics were tested at two time points.

Recent studies of auditory EPs indicated that withdrawal was marked by increased N1-P2 peak-to-peak components, particularly in seizure-prone alcoholics (Neiman et al. 1991; Noldy and Carlen 1990). Similarly, Romani and Cosi (1989) reported larger N1-P2 peak-to-peak components as well as shorter P3 latencies in an auditory oddball paradigm during alcohol withdrawal.

In order to examine whether EP aberrations observed in alcoholics would improve with prolonged abstinence, we examined abstinent alcoholics who were part of a long-term inpatient rehabilitation program (Porjesz and Begleiter 1985). Only alcoholics who were not administered medication were studied at two time points following withdrawal: at 3 to 4 weeks after withdrawal and at 4 months after withdrawal. BAERs and auditory and visual P3s were recorded on both occasions. At the initial testing, we found that BAERs and conduction velocities were delayed. However, following 4 months of abstinence, alcoholics showed improved BAER morphology, shortened latencies, and improved conduction times.

The relative importance of abstinence from alcohol and of nutritional factors in recovery remains undetermined. For example, throughout the long-term treatment in our rehabilitation program, patients received extensive vitamin therapy and most likely improved their nutritional status.

Also, the role of withdrawal cannot be overlooked. CNS hyperexcitability may be

followed by a period of subacute hypoexcitability. This hypoexcitability may be manifested as a prolongation of brain stem latencies caused by aberrant fluidizing effects on the membranes that may result in edema. It has been reported that edema resulting from osmotic stress can lead to demyelination (Feigen and Budzilovich 1978, 1980; Kleinschmidt-Demasters and Norenberg 1981; Lewis 1976; Yates 1976; also see Harper and Kril, chapter 3; Lancaster, chapter 19).

We could examine reversibility only in alcoholics who completed the 4-month treatment program. Importantly, these individuals were those less impaired at initial assessment. Therefore, we cannot conclude that recovery occurs in all alcoholics regardless of degree of initial impairment. It remains to be determined whether recovery occurs as a function of degree of initial impairment, whether greater impairment requires longer recovery time, or whether there is an asymptotic level of reversibility, regardless of recovery time and initial impairment.

Despite the improvement in the BAER with prolonged abstinence, neither ERP morphology nor P3 amplitude improved following 4 months of abstinence in these same alcoholics. The waveforms and decreased P3 voltages to both auditory and visual stimuli were strikingly similar at initial test and retest. There was also no improvement in the differential enhancement of P3 amplitudes on the basis of task relevance to target stimuli. These results suggest that low P3 voltages may not be reversible or may recover more slowly.

Evidence from our laboratory indicates that alcoholics manifest low voltage P3 amplitudes even following extremely prolonged sobriety (Porjesz and Begleiter 1985). We examined recovering alcoholics with 3 to 10 years of sobriety and found that they still exhibited low voltage P3 components, although BAERs were normal. Thus, it appears that some electrophysiological aberrations improve with sobriety, whereas other anomalies do not. As will be discussed in following sections, one likely hypothesis is that abnormalities which fail to improve with sobriety may precede the development of alcoholism and may actually serve as biological markers for or predisposing factors to alcoholism.

FAMILY HISTORY OF ALCOHOLISM AND ERPS

Brain abnormalities observed in alcoholics are generally assumed to be due to the toxic effects of alcohol on the brain, nutritional deficits, or an interaction of alcohol and nutritional-related factors. However, as will be shown in this section, recent evidence suggests that some of these aberrations may antecede the development of alcoholism and may even be related to a genetic predisposition to alcoholism.

Alcoholics

A great deal of interest has been directed to examining the meaning of the diminished P3 voltages observed in alcoholics. Because the P3 component does not appear to recover with prolonged abstinence (see previous section) and its characteristics appear to be genetically determined (Polich and Burns 1987), the

role of chronic alcohol abuse on P3 characteristics has come into question.

Recently, there have been some investigations of the role of family history in determining the amplitude of the P3 component in alcoholics. In our laboratory, we have repeatedly observed that alcoholics manifest significantly lower visual P3 amplitudes than controls. However, the majority of alcoholics in our studies have a positive family history for alcoholism. Therefore, clarifying the relative importance of alcohol versus family history can be difficult. For example, in a recent study we found, as expected, reduced P3 amplitudes in alcoholics (Porjesz et al. 1987*a*). When the alcoholic sample was divided into family history positive (FHP) and family history negative (FHN) subgroups, FHP groups tended to exhibit lower visual P3 amplitudes. However, this difference approached but did not achieve significance (Henry et al. in preparation). In order to be considered FHP in this study, it was only necessary to have an alcoholic father. It is likely that the small sample sizes and having only one alcoholic relative contributed to the nonsignificant results.

Patterson et al. (1987) reported significantly smaller auditory and visual P3 amplitudes in alcoholic males compared to controls. In addition, they found that FHP alcoholics manifested the lowest P3 amplitudes. P3 differences between FHP and FHN alcoholics were significant in the visual modality but only approached significance in the auditory modality. Patterson et al. (1987) attribute their findings to family history. However, they did not eliminate the possible contributions of lifetime drinking history or pattern of alcohol consumption in accounting for P3 amplitude decrements.

Recent evidence from a PATH analysis performed by Pfefferbaum and colleagues (1991) indicates that family history of alcoholism rather than lifetime consumption determines whether alcoholics manifest low P3 amplitudes. They found that FHP male alcoholics had reduced P3 amplitudes for both visual and auditory oddball paradigms compared to FHN alcoholics. P3 amplitude to attended targets was significantly correlated with the number of first-degree relatives with drinking problems for both the auditory and visual RT paradigms. This decreased amplitude was found to be independent of lifetime alcohol consumption in FHP alcoholics.

Thus, there is substantial evidence that reduced P3 amplitudes observed in alcoholics are a function more of family history than chronic alcohol ingestion per se. Perhaps, earlier differences between laboratories regarding P3 findings are, in part, due to differences in the compositions of the alcoholic samples (i.e., the number of FHP versus FHN subjects).

However, it should be noted that all of these studies were conducted using alcoholic subjects. This design makes it difficult to separate the consequences of years of chronic alcohol abuse from other factors. Therefore, a more direct approach to investigating this issue looks at FHP individuals who have not abused alcohol.

Offspring of Alcoholics

Evidence from population genetics studies indicates that sons of alcoholic fathers are

four times more likely to develop alcoholism than are sons of nonalcoholic fathers (Goodwin 1979; Goodwin and Guze 1974). This heightened probability exists even when they are separated from their biological parents soon after birth (Cloninger et al. 1981). Studies of male adoptees in Scandinavia indicate that the biological rather than the adoptive parent is predictive of later drinking problems (Bohman 1978; Cadoret and Gath 1978; Cadoret et al. 1980; Goodwin et al. 1973; Goodwin and Guze 1974). Furthermore, the concordance rate for alcohol abuse between identical twins is almost double the rate for fraternal twins (Kaij 1960), and patterns of alcohol consumption are highly concordant among identical twins (Partanen et al. 1966; Jonsson and Nilsson 1968; Loehlin 1972). Thus, these data, covering several decades of research, suggest that genetic factors predispose sons of alcoholic fathers to alcoholism.

There is a good deal of evidence indicating that characteristics of both the EEG and ERP are also genetically determined. For example, the production of fast EEG activity is genetically transmitted (Vogel 1970; Young et al. 1972; Propping 1977). In various studies, Vogel reported on the hereditary nature of several variants (monomorphic alpha, low voltage EEG, EEG with alpha and beta diffusely mixed, EEG with fronto-precentral beta) (Vogel 1970; Vogel et al. 1986). Vogel maintains that the low voltage and regular alpha EEG are inherited via an autosomal dominant mode, whereas the poor alpha or diffuse beta variants are under polygenic control (Vogel 1970).

In addition to EEG patterns being genetically determined, there is also evidence that ERPs are under genetic control. Monozygotic twins manifest ERP waveforms that are as concordant with each other as EPs obtained from the same individual tested twice (Dustman and Beck 1965; Surwillo 1980). EPs recorded to flashes of different intensities have also been reported to be under genetic control (Buchsbaum and Pfefferbaum 1971). Furthermore, the P3 component of the ERP is more similar in identical twins than in unrelated controls (Polich and Burns 1987).

Given the genetic control of brain electrophysiology, the apparent genetic influence on the development of alcoholism, and the data suggesting alcohol-related abnormalities in brain electrophysiology, it is likely that a genetic predisposition to alcoholism is manifested in brain function. Thus, the study of the offspring of alcoholics, referred to as high-risk (HR) individuals, constitutes an important area of research. HR studies are important because they may provide information regarding preexisting abnormalities in brain electrophysiology that may indicate an increased susceptibility to alcoholism and/or the negative consequences of alcohol on brain function (i.e., alcohol-induced brain damage).

For over a decade, we have been studying ERPs in HR subjects. In our first study, the HR group consisted of 7- to 13-year-old alcohol-naive sons of alcoholic fathers (Begleiter et al. 1984). Their fathers had been diagnosed as alcoholic (DSM-III criteria) and had received prior

treatment for alcoholism. Boys whose mothers either ingested alcohol during pregnancy or who drank excessively after birth were excluded. The low-risk (LR) group was comprised of healthy normal boys matched with the HR group on age and socioeconomic status. LR subjects were included only if they had no first- or second-degree relatives with a history of alcoholism or other psychiatric disorder. Only boys with neither medical problems nor exposure to alcohol or other substances of abuse were included.

A complex visual head-orientation paradigm was used to elicit the P3 component. The target stimulus was a rarely occurring aerial view of the head with the nose and either the right or left ear present, rotated in one of two possible positions (up or down). These targets were interspersed randomly among nontargets (ovals). Subjects were required to press one of two switches to the targets, indicating whether the right or left ear was presented, as quickly and accurately as possible. In the "easy" condition, the head was facing forward (nose up on the screen) and the left or right ear appeared on the same side as the appropriate button. In the "difficult" condition, the head was facing back (nose down on screen) and the left or right ear appeared on the side opposite the corresponding button. P3 amplitudes were significantly smaller in the HR compared to the LR group in response to all target stimuli. This group difference was most obvious at the parietal electrode (where P3 is maximum) for the difficult condition. Principal component analyses with varimax rotation (PCAV) performed on the data indicated that only the factor representing the P3 component was significantly different between the HR and LR groups.

Begleiter et al. (1987*b*) studied another group of sons of alcoholics to determine whether the reduced P3 amplitudes observed in HR subjects were modality or task specific. A modified auditory oddball task was used requiring subjects to press a button in response to rarely occurring tones presented at a random rate; accuracy was stressed over speed. Twenty-three matched pairs of FHP and FHN males between the ages of 7 and 16 were studied. They were carefully interviewed to ascertain that they had no prior exposure to alcohol or illicit drugs. The fathers of the HR boys met criteria for "male-limited," Type 2 alcoholism (Cloninger 1987). Specifically, the fathers indicated early onset of alcoholism and a high rate of recidivism (often accompanied by petty criminality), and required extensive treatment. Additionally, the HR boys came from families with high densities of alcoholism. Extending previous work with visual stimuli, FHP boys exhibited reduced auditory P3 amplitudes. Thus, reduced P3 amplitudes in HR subjects do not appear to be task or modality specific and appear to be present under speed and accuracy conditions.

Another laboratory (Whipple et al. 1988, 1991) used a continuous performance test (CPT) to examine ERPs in prepubescent HR boys. In the first study, they used a visual paradigm consisting of a complex series of visual stimuli that changed along three dimensions–shape,

color, and number. The subject silently counted each time a stimulus identically matched the one preceding it on all three dimensions. In agreement with both Begleiter et al. (1984) and O'Connor et al. (1986, 1987), Whipple et al. (1988) reported a reduction in the amplitude of the late positive complex, including the P3 component. Later studies in the same laboratory have replicated these findings (Noble 1990; Whipple et al. 1991).

We have recently replicated our original findings in an older sample (18 to 23 years of age) of HR male subjects (Porjesz and Begleiter 1990). The sample consisted of 25 male offspring of carefully diagnosed male alcoholics and was selected from high-density alcoholic families (mean number of alcoholic family members = 4). Furthermore, individuals whose mothers abused alcohol before, during, or after pregnancy were excluded. Controls were matched to the sons of male alcoholics on the basis of age, education, and socioeconomic status. Controls were selected from families with no history of alcohol abuse or alcoholism in either first- or second-degree relatives. FHP and FHN subjects were carefully matched on drinking history, including duration and quantity–frequency information.

In this study, we used the previously described visual-spatial paradigm involving easy and difficult line discriminations (see section on P3 in alcoholics for description). The results indicated that P3 amplitude was significantly lower in HR subjects compared to controls. This pattern replicates our previous findings (Begleiter et al. 1984, 1987*b*) with an older sample of HR subjects and also replicates the work of O'Connor et al. (1986, 1987) and Whipple et al. (1988, 1991). The largest differences in P3 amplitude between the groups occurred in response to the easy target, to which the LR groups produced extremely large P3s. These results parallel those obtained in alcoholics using the same paradigm (Porjesz et al. 1987*a*). This P3 amplitude difference between groups was most apparent at Pz and Cz electrodes.

Most recently, using another auditory target selection task, we observed that adolescent HR males manifest lower amplitude P3s than LR males (Porjesz and Begleiter in preparation *a*). In this paradigm, rare or frequent tones were randomly presented rather quickly (600 to 800 milliseconds) to either the right or left ear. The rare tones to a specific ear were designated as targets, and the subject pressed a button in response to these as quickly as possible. The same rare tones to the other ear were ignored.

In the absence of other differences between groups (N1 amplitude), HR males showed lower amplitude P3 components to both the rare attended (P3b) and unattended (P3a) tones. These findings indicate that HR subjects did not make probability matches as well as LR subjects. In an inattention auditory oddball paradigm, P3a amplitude was also reduced in HR adolescent males. In this experimental design, subjects read a book during the binaural presentation of rare and frequent tones.

In summary, this literature indicates that P3 is reduced in HR males in response to both attended and unattended

stimuli, and in response to both easy and difficult discriminations in both visual and auditory modalities. Despite the general consensus that P3 amplitudes are lower in HR males, some studies such as those conducted by Polich and Bloom (1987, 1988) and Baribeau et al. (1987) have failed to replicate these findings.

Baribeau et al. (1987) examined HR and LR subjects who were further subdivided according to amount of alcohol consumed (heavy versus light drinkers). They used an auditory selective attention paradigm in which rare (500 Hz) and frequent (600 Hz) tones were randomly presented to either the right or left ear at a random rate (630 to 880 milliseconds). Subjects were instructed to count the signals in one ear and ignore those in the other ear.

HR subjects in this study did not exhibit reduced P3 amplitudes. However, the light drinkers in the HR group manifested smaller (though not significantly smaller) P3s in the inattention condition. These results suggest that when attention is mobilized, P3 deficits are not apparent in the attended channel. Perhaps the reduction of P3 amplitude in the unattended channel would reach significance with a larger number of subjects. As mentioned previously, we have found reduced P3 amplitudes in response to rare tones in the unattended channel in HR subjects with a paradigm similar to this one.

In this same study (Baribeau et al. 1987), HR subjects exhibited significantly larger N1 components than did LR subjects in the attention condition. This finding may indicate that the HR subjects in their study paid more attention to the stimuli than did the LR subjects, perhaps because the tone discrimination was perceived as being more difficult to the HR subjects.

It is important to note that the subject sample in this study represents an older group of HR individuals. There is a rather large age range (19 to 35) with mean ages of 27 (HR-heavy drinking), 22 (HR-light drinking), 24 (LR-heavy drinking), and 25 (LR-light drinking). These HR subjects may have passed the age of risk, rendering the sample unrepresentative of groups at high risk for alcoholism. This observation may be particularly applicable because those who already manifested alcoholic problems were excluded. If by this age they have not developed alcohol-related problems or become alcoholic, the likelihood is that they will not. Thus, this group may represent a skewed sample of HR subjects, perhaps endowed with protective mechanisms. Certainly, their larger N1 component suggests they are atypical.

Related to the issue of possible protective mechanisms, it is interesting that Hill et al. (1988) reported increased cognitive efficiency in nonaffected siblings of alcoholics. They observed shorter P3 latencies in these nonaffected siblings and suggested this finding reflected some protection against the development of alcoholism in so-called HR subjects.

A number of studies using college students with positive family histories of alcoholism have been conducted by Neville and colleagues (Elmasian et al. 1982; Neville and Schmidt 1985; Schmidt and Neville 1985) and Polich and colleagues (Polich and Bloom 1986, 1987,

1988; Polich et al. 1988; Schuckit et al. 1988) at the University of California at San Diego. These studies have produced interesting yet conflicting results.

Following the administration of either alcohol or a placebo, differences in P3 characteristics have been found between HR and LR subjects. Elmasian et al. (1982) studied the P3 component of the ERP in HR and LR male college students (ages 20 to 25) under placebo and low and high doses of alcohol. After alcohol or placebo administration, they reported significant P3 amplitude decreases in the HR compared to the LR subjects. Elmasian et al. (1982) explained their results in terms of differential expectancies for alcohol characterized by different brain events. The investigators also suggested that the results may be due to higher than normal alcohol intake in the mothers of the HR subjects. Unfortunately, different sets of subjects were used for each dose, and there were only five pairs of subjects per group. Therefore, accurate interpretation is difficult. Interestingly, the placebo effect was not replicated in later work in this same laboratory (Polich and Bloom 1988).

In a another study from the same laboratory (Neville and Schmidt 1985), the late positive component (LPC) of the ERP in HR individuals was investigated without the ingestion of any liquid. In this study, mothers of all subjects were interviewed with respect to their alcohol and drug use, and the experimental design eliminated expectancy effects. Group differences in the LPC were still obtained.

In another study, Schmidt and Neville (1985) investigated ERPs in HR males while they were engaged in a visual language task. They found that the N430 component (the component related to semantic processing; see section on N400) was significantly smaller in the HR men than in LR men. Moreover, in the HR group the latency of N430 was directly related to the amount of alcohol consumed per occasion. These fascinating results imply that neuronal function associated with language processes are affected by family history, and that there is an interaction between family history, alcohol consumed, and N430. We are currently examining HR subjects with a semantic priming paradigm.

Polich and Bloom (1987, 1988) and Schuckit et al. (1988) did not find P3 amplitude differences between groups of FHP and FHN male college students. Using an auditory oddball paradigm, Schuckit et al. (1988) did not find any ERP differences between FHP and FHN prior to ethanol ingestion or following a placebo dose. Following a high dose of ethanol (1.1 mL/kg), P3 latency delays returned to baseline measures more rapidly in FHP men. This finding suggests that some electrophysiological differences between FHP and FHN individuals are apparent only in response to ethanol challenges. These differences in response to ethanol challenge may represent an innate tolerance in the FHP subjects.

An inverse correlation between the amount of alcohol consumed (drinks per sitting) and the amplitude of P3 was found by Polich and Bloom (1987) without the administration of alcohol. However, this relation was apparent only

for a difficult auditory intensity discrimination task in FHP subjects. Although there was a trend in this direction for FHN subjects, it was not significant. The authors concluded that FHP subjects are more sensitive to the effects of alcohol than FHN subjects. When a similar intensity discrimination study was performed in the visual modality, no correlation between P3 characteristics and amount of alcohol typically consumed was found (Polich et al. 1988). However, in a later study designed to replicate Elmasian et al. (1982), Polich and Bloom (1988) not only failed to replicate the placebo effect, but reported a correlation between P3 latency and amount of alcohol consumed for both FHP and FHN subjects.

The relation between P3 characteristics and drinking history is as yet an unresolved issue in other laboratories as well. O'Connor et al. (1986) reported no relation between any P3 characteristic and drinking history. However, Steinhauer et al. (1987) did obtain a correlation between P3 latency and drinking history.

One possible explanation for the lack of significant results from the San Diego groups involves the mode of assessment of alcoholism in the fathers and the clinical assessment of their families in general. A questionnaire is completed by the son about his father's and first- and second-degree relatives' alcohol and psychiatric history. To be scored as FHP, only one positive symptom regarding the father's alcoholism is required. Thus, it is possible that in a large percentage of subjects, the offspring are not offspring of alcoholics but rather of heavy or moderate drinkers. This classification procedure weakens the possibility of obtaining ERP differences between FHP and FHN groups. Therefore, it is conceivable that there is more agreement in the literature dealing with subjects at risk for alcoholism than had been heretofore suspected.

Although it has been hypothesized that discrepancies in results between laboratories may be due to task difficulty, recent evidence fails to support this contention. O'Connor et al. (1987), using two tasks at different levels of task difficulty, obtained identical results with both paradigms. Begleiter and his colleagues replicated their finding of a lower P3 amplitude in HR subjects without ingestion of alcohol in four different paradigms thus far. Those used are a complex visual response-compatibility/incompatibility design (Begleiter et al. 1984), an auditory modified oddball paradigm (Begleiter et al. 1987*b*), a visual discrimination paradigm (Porjesz and Begleiter 1990), and an auditory Hillyard paradigm (Porjesz and Begleiter in preparation *b*). It is important to note, however, that task difficulty is not necessarily a continuum along which P3 results can be explained. Some aspects of task difficulty alter P3 characteristics, whereas others do not. For example, difficulty of stimulus discrimination alters P3 characteristics, but response selection does not.

We have recently investigated the effects of alcohol on visual ERPs in HR and LR subjects (Porjesz and Begleiter 1992). Twenty-four pairs of male HR and LR subjects between the ages of 19 and 24 received either a placebo or one of two

alcohol doses (0.5 mL/kg or 0.8 mL/kg). The visual ERP paradigm involving easy and difficult line discriminations described earlier was used. ERPs and measures of levels of intoxication were obtained prealcohol and at 20, 60, 90, and 130 minutes following alcohol ingestion. Blood alcohol levels (BALs) were monitored at 10-minute intervals throughout the session. No significant differences were obtained between groups in terms of BALs or intoxication ratings.

As reported previously, the P3 amplitude was reduced in HR subjects relative to LR subjects to all target stimuli, but particularly to the easy target, prior to alcohol consumption. Alcohol ingestion did not affect the difference in amplitude between groups. Although there was a tendency for alcohol to depress the amplitude of P3 in both groups, this depression did not achieve statistical significance.

However, during the ascending phase of the BAL, the HR group showed a larger percent decrement in P3 amplitude than the LR group to both target stimuli. This pattern may indicate greater sensitization in the HR group on the ascending phase of the BAL (Newlin and Thomson 1990). Similarly, we found more of an increase in slow alpha activity on the ascending limb, indicating sensitization, in HR subjects following an alcohol challenge (Cohen et al. in press).

No significant difference in the latency of P3 occurred between groups prior to alcohol ingestion. The latency of P3 occurred significantly later in response to the difficult compared to the easy discrimination target in both groups. The high dose of alcohol increased the latency of P3 to the difficult target in both groups at all but occipital electrodes. This effect was maximal between 60 and 90 minutes postalcohol, that is, at peak and early descending BALs. Although the HR and LR groups did not differ in terms of initial alcohol-induced P3 latency delays, the HR group appeared to recover more quickly to prealcohol ranges. This finding replicates the work of Schuckit et al. (1988) who reported that FHP males recover more quickly from alcohol-induced P3 latency delays.

The N1 amplitude was significantly decreased by alcohol ingestion beginning at 20 minutes, particularly for the nontarget stimuli at occipital electrodes. This result was more pronounced for the LR than the HR group. Although the N1 to nontargets remained depressed in the LR group throughout the test, it recovered by 90 minutes in the HR group. These results suggest that the HR subjects exhibited an innate tolerance to alcohol, as compared to the LR group. Under alcohol conditions, the N1 amplitude was only partially reduced in response to the easy target and did not decrease in response to the difficult target.

These results support the finding by Roth et al. (1977) that attentional factors can counteract the alcohol-related decreases in N1, and the findings by Campbell and Lowick (1987) that the largest alcohol effects were obtained when attention was least mobilized (nontarget conditions).

The differential effect of alcohol on N1 is an important difference between HR and LR groups and parallels the behav-

ioral results reported by Schuckit et al. (1988). These results suggest that HR subjects exhibit more acute tolerance than LR subjects. Whereas there was a tendency for HR subjects to drink more frequently than the LR subjects and to consume more alcohol per sitting, neither of these differences reached statistical significance. However, we cannot conclude whether this N1 effect is due to innate or acquired tolerance.

This literature indicates that ERPs provide sensitive indices of state and trait variables involved in alcoholic consumption and that specific components of the ERP are differentially sensitive to various aspects of alcohol-related effects. Additional research must be conducted to determine whether subjects with low P3 amplitude before alcohol ingestion also manifest less N1 response to alcohol, and whether these individuals are in fact at higher risk for alcoholism.

Thus, an important question concerns the identification of which differences in electrophysiological function antecede alcoholism and which differences are consequences of years of heavy alcohol consumption. To address this question, we have investigated in nonalcoholic sons of alcoholics many different EP characteristics that are aberrant in chronic alcoholics. In one study discussed earlier regarding P3 changes (Begleiter et al. 1987*a*), we also assessed BAERs in LR and HR subjects. In contrast to the P3 findings, we did not observe any significant differences in BAER measures between LR and HR subjects. Thus, BAER abnormalities observed in alcoholics appear to be consequences of chronic abuse, whereas the P3 amplitude differences appear to be independent of alcohol consumption and may represent trait differences. This finding is underscored by the recovery of the BAER but not the P3 with prolonged abstinence. We are currently examining in HR subjects other ERP components that are aberrant in alcoholics (e.g., MMN, P3a, N400, and VMP).

As noted earlier, the lack of consensus of results among laboratories may at least in part be attributed to differences in subject populations. The only definition of risk for alcoholism that these studies share is that at least the father must have been "alcoholic." Therefore, the density of alcoholism within the family fluctuates across studies. If only the individual's father and no other first- or second-degree relatives are alcoholic, this may not increase the genetic risk for alcoholism but may indicate a phenocopy or sporadic case. Furthermore, the clinical criteria for diagnosis of alcoholism in the father and the manner in which his alcoholism is assessed contribute to differences in the samples studied. Some studies require only one symptom of alcoholism in the father to qualify for inclusion into the FHP group. Therefore, the HR groups in some studies may include offspring of nonalcoholic but heavy or problem drinkers. As previously mentioned, this broad criterion weakens the loading of familial alcoholism and makes it less likely that significant differences between groups will be obtained.

Problems such as comorbidity for other psychiatric problems are also treated

differently in various studies and may contribute to the disparate results. Individuals manifesting comorbid psychiatric diagnoses (e.g., antisocial personality or affective disorder) may be excluded from some studies yet be included in others.

Because alcoholism is a heterogeneous disease, HR groups in different studies may be composed of varying numbers of offspring of different "types" of alcoholism (e.g., Type 1, Type 2). This mixing of types may complicate outcomes because various types of prealcoholic offspring may manifest different electrophysiological patterns before and/or after alcohol administration.

Often, the HR subjects examined are beyond the age of risk, or the stringent screening criteria rule out potential prealcoholics. This selection procedure results in so-called "high risk" subjects who may actually be at low risk for developing alcoholism.

Selection criteria as applied to control subjects must also be carefully examined when comparing the results of these studies. Finally, subject variables such as age, education, and socioeconomic status may influence outcome in both HR and control samples and must be examined to eliminate possible confounds.

Obviously, subject selection remains a major problem in HR research. Ideally, the HR sample consists of young children without prior exposure to alcohol who are offspring of alcoholic fathers from families in which alcoholism is prevalent; these alcoholic fathers should be diagnosed directly.

SUMMARY AND CONCLUSIONS

Electrophysiological research using a variety of paradigms has revealed that a number of ERP components (i.e., N1, P3, N2, MMN, N400, and VMP) are aberrant in alcoholics under certain conditions. Each of these ERP components is sensitive to different aspects of information processing. However, when reviewed as a whole, the literature suggests that alcoholics do not electrophysiologically differentiate between relevant and irrelevant, target and nontarget, easy and difficult, primed and unprimed, familiar and unfamiliar, or even same and different stimuli. Indeed, they maintain the same ERP characteristics (both amplitude and latency) regardless of stimulus or task requirements.

Unfortunately, the neural origins of most ERP components are not known. Therefore, it is difficult to identify which brain areas of alcoholics are most compromised using this technique. Increasing evidence indicates that multiple brain areas contribute to scalp-recorded ERPs. Given the neuropsychological data suggesting involvement of the frontal lobes, it is tempting to speculate that the common component of these ERPs originates in frontal areas. Indeed, as reviewed there is evidence implicating frontal contributions to P3. Additionally, the MMN is thought to have contributions from frontal areas. Furthermore, despite its origins in inferior temporal regions, recent work from our laboratory indicates frontal contributions to the P240 component. Certainly from a theoretical and rational level, the match-mismatch processes themselves (e.g., the ability to differentiate relevant

from irrelevant) appear to be a frontal function.

Future work must continue the focus of separating those brain aberrations that antecede alcohol abuse from those reflecting years of heavy drinking. The delineation of specific neurophysiological deficits in abstinent alcoholics and children at risk for alcoholism may be of fundamental importance in the identification of possible genetic marker(s) for differential responsiveness to alcohol and/or the development of alcoholism per se.

However, to accomplish these goals, long-term longitudinal studies are needed to assess HR individuals as they pass through the age of risk. At present, there is no compelling evidence that subjects showing reduced P3 amplitude are, in fact, destined to become alcoholics. Longitudinal family studies are underway and, hopefully, these studies will elucidate the link between electrophysiological aberrations and the development of alcoholism. Finally, although it is increasingly obvious that genes are an important component in the development of certain types of alcoholism, the role of environment and its interaction with genes cannot be overemphasized. Comprehensive studies must address all of these factors if we are to more completely understand the etiology of and consequences of alcohol abuse.

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6

FRONTAL LOBE CHANGES AFTER CHRONIC ALCOHOL INGESTION

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INTRODUCTION

Generalized diffuse cortical atrophy has been associated with long-term chronic alcoholism. In alcoholic Korsakoff's syndrome, there is extensive subcortical damage as well. Brain systems implicated in the complex functions disrupted by chronic alcohol abuse include the limbic lobe, diencephalon, basal forebrain, and neocortical regions (Arendt et al. 1983; Barbizet 1970; Cala et al. 1978; Harper and Kril 1990; Horel 1978; Jernigan et al. 1991*a*,*b*; Lishman 1990; Wilkinson and Carlen 1982). The region of the cerebral cortex that appears most consistently affected by alcoholism is in the frontal lobes, although magnetic resonance imaging (MRI) scans and post mortem studies have implicated the temporal and parietal lobes as well (Courville 1955; Harper and Kril 1990; Jernigan et al. 1991*a*,*b*). Alcoholics, especially patients with Korsakoff's syndrome, exhibit clinical signs associated with damage to the frontal cortex, including emotional apathy, disinhibition, poor attention, confabulation, and abnormal response perseveration (for reviews, see Oscar-Berman 1992; Walsh 1991).

Several hypotheses have been proposed to explain the diverse neuropsychological deficits exhibited by chronic alcoholics. These hypotheses are not necessarily mutually exclusive, and discussions or reviews of key hypotheses and theories can be found in papers by Bowden (1990), Ellis and Oscar-Berman (1989), Lishman (1990), Oscar-Berman et al. (1991), Oscar-Berman (1992), and Walsh (1991), among others (see also Nixon, chapter 10). The hypotheses stress the involvement of different brain systems, as follows: (1) Alcoholics have diffuse cortical damage that disrupts the functions of both hemispheres; (2) chronic alcohol abuse leads to dysfunctions associated with damaged frontal–diencephalic systems of the brain; (3) alcoholism leads to selective impairments in right hemisphere functions; and (4) chronic alcohol abuse results in premature aging of the brain. Considerable evidence

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both supports and refutes each of these hypotheses. However, most studies clearly suggest that alcohol abuse results in diffusely distributed brain damage, with special emphasis on damage to the frontal–diencephalic systems.

This chapter explores evidence consistent with the idea that prefrontal cortical pathology contributes to behavioral aberrations in alcoholics, with and without symptoms of Korsakoff's disease. A brief description of neuropsychological changes associated with human frontal lobe damage unrelated to alcoholism is followed by a review of deficits in monkeys with frontal lesions. An appraisal of the neuroanatomical subsystems of the frontal lobes reveals at least two parallel subdivisions having relevance for two distinct sets of neurobehavioral functions. There is evidence that both of the subsystems may be damaged after prolonged alcohol ingestion, and that the degree of involvement is reflected in the behavioral manifestations of alcoholism.

FUNCTIONAL CHANGES WITH DAMAGE TO FRONTAL LOBE SYSTEMS IN HUMAN AND NONHUMAN PRIMATES

For more than a century, the frontal lobe syndrome has remained an enigma. In humans, the syndrome consists of dramatic changes in personality, as well as elusive cognitive changes. Changes in personality have been described in terms of "disinhibition" and lack of concern for the consequences of strange behaviors. A previously mild-tempered, responsible individual may, for example, become irresponsible, profane, easily provoked, and show poor judgment (Benton 1991; Luria 1980; Rylander 1939; Walsh 1987). In comparison to the often dramatic personality changes, intellectual or cognitive changes are mild but unmistakable. Indeed, only one facet of cognitive change has stood out consistently, that is, abnormal perseverative responding (Lezak 1983; Lhermitte et al. 1986; Stuss and Benson 1986; Teuber 1955; Valenstein 1980). The abnormality is one of response inhibition, where the affected individual is unable to stop repeating an ongoing behavior. It is especially marked on tests of problemsolving, abstract reasoning, attention, imitation, motor control, spatial scanning, and sequencing. Abnormal perseverative responding has been linked to dysfunctions having numerous labels, including working memory (Goldman-Rakic 1992), mediating cross-temporal contingencies (Fuster 1989), and executive functioning (Lezak 1983; Lhermitte et al. 1986; Stuss and Benson 1986).

In nonhuman primates, the most salient consequence of bilateral frontal lobe damage is failure to perform normally on a class of spatial tests known as delayed-reaction tasks (Fuster 1989; Oscar-Berman et al. 1991; Stuss and Benson 1986; Warren and Akert 1964). Deficits on delayed-reaction tasks also have been demonstrated in humans with bilateral prefrontal damage (Freedman and Oscar-Berman 1986*a*; Pribram et al. 1962). Delayed-reaction tasks refer generally to a class of laboratory procedures employed to measure mediation ability (the ability to bridge a time gap). Intact mediation abili-

ty is inferred from accurate choices made in the present, based on information that was available in the immediate past. The simplest type of delayed-reaction test is the direct-method or classical delayed-response (DR) task. In this task (see figure 1), the experimenter puts a piece of food into a reinforcement well under one of two identical stimuli, for example, two black wooden plaques differing only in their spatial location. The research participant (in this case, a hungry monkey) sees the food being placed under one of the plaques but cannot reach it. When the wells are covered by the stimuli, a screen comes down between the experimenter and the subject, and a delay period begins. Typically, the delay lasts between 0 and 60 seconds, during which time the monkey has to remember where the food had just been placed. When the delay ends, the screen comes up again, and the tray with the hidden food is now within the monkey's reach. If the monkey knows where the food is, the monkey will push one of the stimuli away and retrieve the food. If the monkey chooses the wrong side, it gets nothing. Sometimes an indirect cueing method is used, in which a stimulus such as a light or sound signals the location of the reinforcement (Oscar-Berman 1975, 1978). The DR task is time based in that

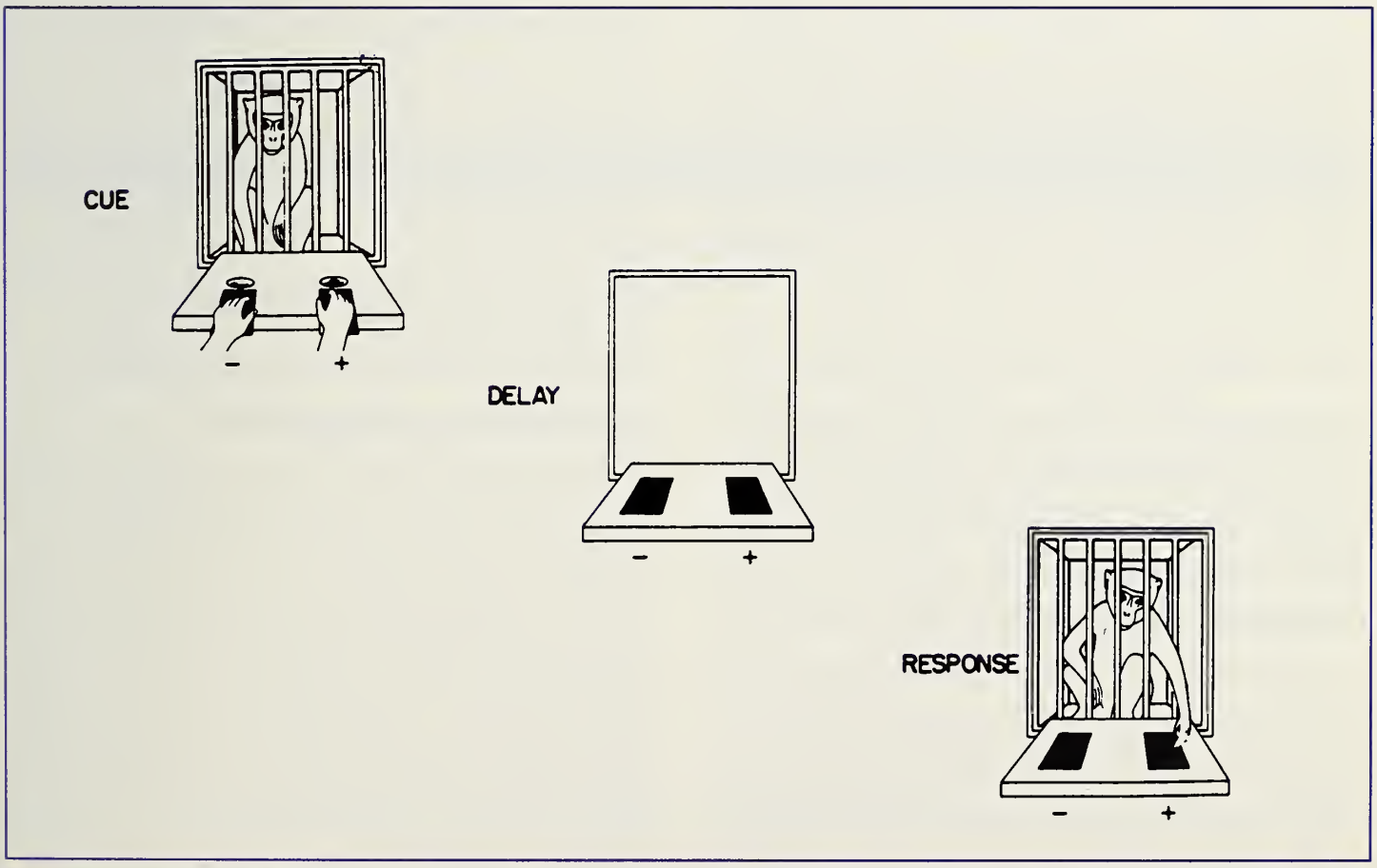

FIGURE 1

Three parts of a classical direct-method delayed-response trial. A reward is placed into a well under one of two identical stimuli differing only in spatial location. As soon as the wells are covered by the stimuli, a screen is lowered between the experimenter and the subject. After a delay (usually between 0 and 60 seconds), the screen is raised with the tray containing the stimuli within the subject's reach. Reproduced with permission from Oscar-Berman et al. (1991).

the subject must delay a choice until the opportunity to respond is made available. This version of the task is not response patterned because a regular sequence of responding is irrelevant to obtaining the reinforcements.

Another type of delayed-reaction task, the delayed-alternation (DA) task, is both time based and response patterned. DA tasks are usually given in an experimental apparatus identical to that used for measuring DR performance. Certain important features are common in DR and DA tasks. Specifically, these commonalities include (1) a delay between the stimulus and the opportunity to make a response and (2) a spatial cue as a salient stimulus dimension. However, the task requirements in DA and DR are quite different. In DA, the subjects must learn to alternate responding from left to right. On each trial, a response to the side not previously chosen is rewarded, and a brief delay is interposed between trials, usually 5 seconds, with the screen lowered between the experimenter and the subject. Thus, in the classical DR task, the subject utilizes spatial–attentional and mnemonic skills to notice and remember an external cue. In the DA task, the sources of cues are not so conspicuous; the cues about the locus of the reinforcement can originate inside the subject (e.g., where the subject last responded) and/or outside the subject (whether or not a reward was available). In any case, on each trial, the subject must learn to inhibit the previously rewarded response.

Several explanations have been offered regarding the nature of DR and DA deficits. None, however, has proved entirely satisfactory. Explanations generally include abnormalities in the following functions: short-term memory (e.g., episodic memory, working memory, and immediate memory), spatial information processing, response inhibition (and its corollary, perseveration), temporal chunking, and kinesthetic feedback (for reviews, see Arnold 1984; Damasio 1979; Fuster 1989; Goldman-Rakic 1987; Pribram and Tubbs 1967; Stamm 1987; Stuss and Benson 1986). Despite a lack of consensus regarding the precise nature of the functions being tapped by DR and DA tasks, they are universally assumed to be sensitive measures of one or more cognitive functions linked to neuroanatomical systems of prefrontal cortex. In addition, successful performance on the tasks requires an ability to mediate cross-temporal contingencies (i.e., bridge a time gap).

NEUROANATOMY OF FRONTAL LOBE SYSTEMS IN HUMAN AND NONHUMAN PRIMATES

Since the mid-1930's, when Jacobsen initially described selective DR deficits in monkeys with lesions of prefrontal cortex (Jacobsen 1936), the impairment has been a hallmark of the damage (Warren and Akert 1964). Two large subdivisions of prefrontal cortex have been recognized as important in normal DR and DA performance (see figure 2): the dorsolateral and polar extent of the frontal lobes (especially area 46 in the principal sulcus), and the ventral prefrontal region including the orbitofrontal surface and inferior convexity (Barbas and Pandya 1991; Mishkin and

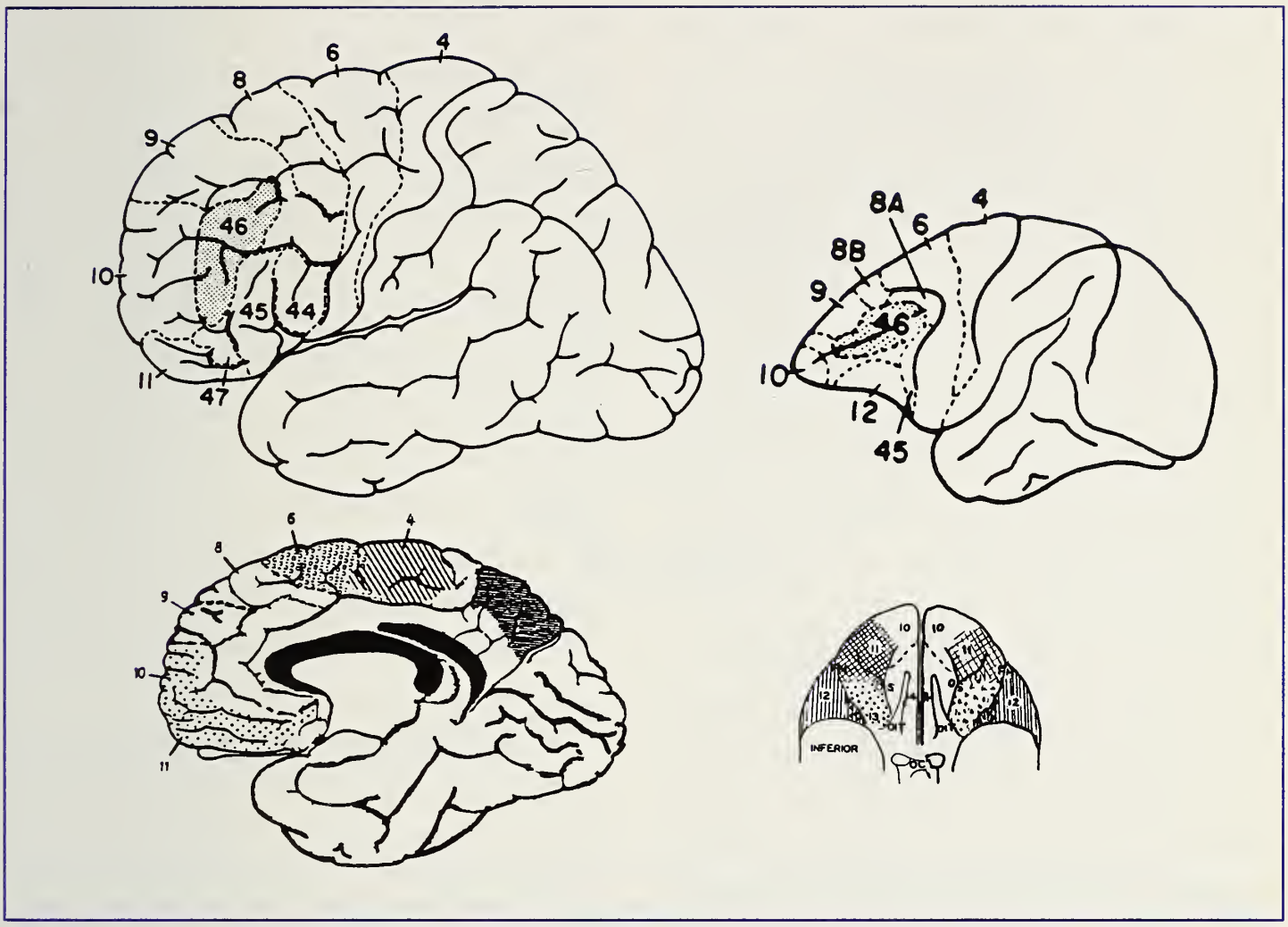

FIGURE 2

Cytoarchitectonic areas of a human brain (Brodmann 1909) and the brain of a Rhesus monkey (Walker 1940). Reproduced with permission from Oscar-Berman et al. (1991).

Pribram 1956; Numan 1978; Oscar-Berman 1975; Rosenkilde 1979; Stuss and Benson 1986; Warren and Akert 1964).[3]

The dorsolateral and ventral subdivisions of prefrontal cortex have different cytoarchitectonic characteristics, neurochemical sensitivities, and connections with the rest of the brain (see figure 3). The dorsolateral system maintains more intimate connections with other neocortical sites than the ventral system. However, the dorsolateral system's connections with limbic sites are less striking than the orbitofrontal system's. Also, although the dorsolateral system is important for successful performance on both DR and DA, it is especially important in DR. Visuospatial memory and attentional functions are thought to be compromised with dorsolateral lesions.

By contrast, functions involved in response inhibition have been linked to orbitofrontal cortex. The ventral frontal system, which includes the orbitofrontal cortex, is intimately connected with basal forebrain and limbic structures. Its con-

[3] *Other subdivisions such as premotor cortex, supplementary motor cortex, and the medial prefrontal sector containing the anterior cingulate gyrus are not included because of little evidence linking those regions directly with functions relevant to the present chapter (Goldman-Rakic 1987; Pribram 1973).*

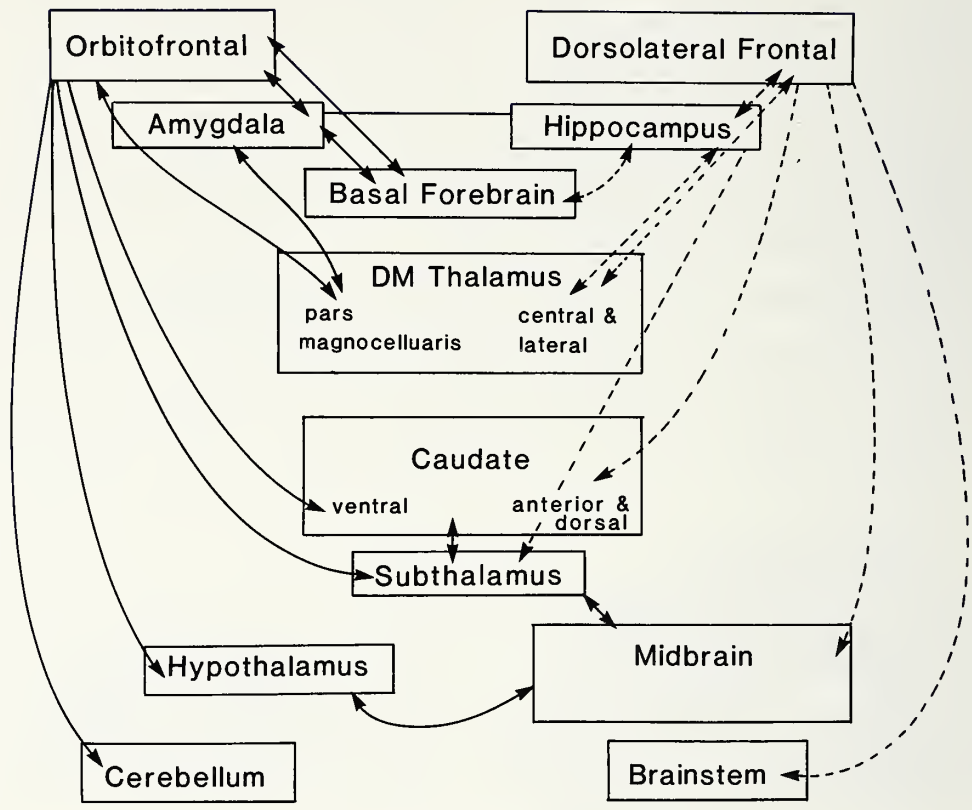

FIGURE 3

Schematic representation of two major prefrontal neuroanatomical systems. Reproduced with permission from Oscar-Berman et al. (1991).

nections with other neocortical regions are not as extensive as the dorsolateral system's. Similar to the dorsolateral system, the ventral system supports successful performance on both DA and DR. It is especially important, however, for DA performance. It should be emphasized that the dichotomy is one of degree and relies upon relative—not absolute—differences in the neuroanatomy, neurochemistry, and behavioral functions of the two prefrontal subsystems.

The original work on behavioral and neuroanatomical systems involved in DR and DA performance was based upon nonhuman primate models. In humans, evidence regarding a prefrontal functional separation along dorsolateral and ventral dimensions is not as convincing as it is with monkeys. One important reason for this is that many different and complex procedures have been used to assess the deficits.

In our own attempts to clarify the functional significance of human prefrontal cortex, we have supplemented standard neuropsychological evaluations with DR and DA tasks. We chose DR and DA specifically because of their special sensitivity to frontal dysfunction in nonhuman primates (but see also Freedman 1990). Furthermore, the monkey literature lends considerable support to the hypothesis that certain aspects of perfor-

mance on DR tasks are mediated by different neuroanatomical (and neurochemical) systems than performance on DA tasks (Oscar-Berman et al. 1991). Consistent with this literature, frontal damage in humans appears to result in abnormalities associated with visuospatial mnemonic and attentional functions (disrupted by damage in the dorsolateral–prefrontal system) or with functions involved in response inhibition (disrupted by damage in the orbitofrontal system). Results of standard clinical neuropsychological evaluations support the dichotomy.

Dorsolateral Prefrontal System

In monkeys and in humans, there are between 6 and 10 cytoarchitectonically distinct areas, respectively, within the dorsolateral prefrontal region (Brodmann 1909; Walker 1940). In monkeys, the dorsolateral region tied most directly to normal performance on spatial-delay tasks lies within the principal sulcus of the dorsolateral surface [Walker's (1940) area 46; the corresponding cytoarchitectonic region in humans is Brodmann's (1909) area 46]. In monkeys, lesions of the principal sulcus (area 46) produce deficits on DR and DA tasks as severe as those involving the entire dorsolateral prefrontal convexity (for reviews see Butters et al. 1972; Fuster 1989; Goldman-Rakic 1987; Pribram 1987; Stamm 1987; Stuss and Benson 1986; Warren and Akert 1964).

Dorsolateral prefrontal cortex has reciprocal projections to and from other neocortical association cortices, limbic structures (via the cingulate cortex and ventral prefrontal cortex), and diencephalic regions (Barbas and Pandya 1991; Fuster 1989; Goldman-Rakic 1987; Pandya and Barnes 1987; Stuss and Benson 1986). It has nonreciprocal efferent connections with basal ganglia sites, sending fibers to the anterior part of the head of the caudate nucleus (Johnson et al. 1968). Lesions of separate structures within the dorsolateral prefrontal system lead to DR deficits, such as the head of the caudate (Battig et al. 1960), the dorsomedial nucleus of the thalamus (Isseroff et al. 1982; Schulman 1964), and the subthalamus (Adey et al. 1962). Mild DA deficits can be produced by lesions of those same subcortical sites important for DR performance. In addition, DA performance is mildly sensitive to damage of the hippocampus, with and without the amygdala (Mahut 1971; Rosvold and Szwarcbart 1964); inferotemporal cortex (Mahut and Cordeau 1963); cingulate cortex (Pribram et al. 1962); and the hypothalamus (Holmes et al. 1983; Rosvold 1972). These findings show that lesions in sites with prefrontal connections can mimic the cortical effects themselves.

Ventral Prefrontal System

The orbitofrontal sector of ventral prefrontal cortex has been associated more often with deficits on DA tasks than on DR tasks (Fuster 1989; Goldman-Rakic 1987; Oscar-Berman 1975; Warren and Akert 1964). Orbitofrontal cortex displays a pattern of connections paralleling that of dorsolateral cortex, but the two systems are distinctly different. Primary orbitofrontal connections are with the medial thalamus (the magnocellular

region of the dorsomedial nucleus), the hypothalamus, the ventrolateral portion of the head of the caudate, and the amygdala.[4] In general, compared to dorsolateral cortex, orbital cortex is (1) more densely interconnected with limbic sites (Mishkin 1964; Nauta and Domesick 1982) and with the basal forebrain (e.g., the medial septal area, the diagonal band of Broca, and the nucleus basalis of Meynert; Mesulam et al. 1983), and (2) less interconnected with other neocortical association areas (Eslinger and Damasio 1985; Pribram 1987).

Cormier (1986), noting functional and neuroanatomical diversity within both the dorsolateral system and the orbitofrontal system (in monkeys, dogs, cats, and rats), separated the two systems according to their roles in complex behavior. Barbas and Pandya's (1991) findings of architectonic differentiation and topographical interconnections of prefrontal regions with other brain sites support such a functional distinction. According to Cormier, of the three major functions of the brain (biological homeostasis, motoric output, and information processing), it is a subdivision of the information processing mechanism, called behavioral analysis, that depends upon intact frontal–reticular interactions (whereas primary sensory and sensory association areas deal with sensory information and its stored representations). The behavioral analysis division of the information processing mechanism has two interacting parts, corresponding to the dorsolateral (habituative) prefrontal system, and the orbital (reinforcer modulation) prefrontal system, respectively. The two prefrontal systems together serve to connect motivationally significant stimuli with appropriate behaviors (dorsolateral regions) while inhibiting responses to stimuli with little or no motivational significance (orbital regions). With language, humans extend the effectiveness of frontal mechanisms by increasing cue control, responses, and planned behaviors.

Conclusions

In summary, while the dorsolateral and ventral prefrontal systems have many parallel connections within the brain, there are important differences between them. The dorsolateral prefrontal cortex is predominantly and reciprocally interconnected with other neocortical association areas (directly or via the diencephalon) and nonreciprocally connected with striatal and brain stem sites. Also, it appears to play a role in monitoring potentially relevant environmental cues and responses. Orbitofrontal cortex is intimately connected with hypothalamic, limbic, and basal forebrain sites and may modulate the effects of reinforcement.

EVIDENCE OF ALCOHOL-RELATED FRONTAL LOBE PATHOLOGY

A lack of consensus exists among researchers regarding which regions of the brain are damaged in Korsakoff and non-

[4] *Dorsolateral cortex connects with a more lateral region of the dorsomedial nucleus of the thalamus (as well as to ventral and anterior thalamic areas), the anterolateral portion of the head of the caudate, the hippocampus, and many neocortical regions (Fuster 1989).*

Korsakoff alcoholics, and whether the regional patterns of damage are distinctly different in these two disorders (see also Cermak, chapter 7 for animal study). Whereas evidence from neuroradiological, neuropathological, and functional imaging studies suggests that chronic alcohol abuse results in diffuse pathology involving many cortical and subcortical regions, much of it supports the idea that damage to frontal lobe systems is predominant. To explain different patterns of damage observed in alcoholics with and without Korsakoff's syndrome, some researchers have proposed that thiamine deficiency primarily causes diencephalic damage. On the other hand, alcohol neurotoxicity primarily causes cortical atrophy, as well as undetected liability of basal brain regions (Lishman 1990; Shimamura et al. 1988).

According to this view, alcoholics who are susceptible to alcohol toxicity alone may develop permanent or transient cognitive deficits associated with cortical shrinkage. Those who are susceptible to thiamine deficiency alone will develop a mild or transient Korsakoff state, with anterograde amnesia as a salient feature. Individuals with dual vulnerability, suffering from a combination of alcohol neurotoxicity and thiamine deficiency, will have widespread damage to basal brain structures as well as to large regions of the cerebral cortex. These people will have severe anterograde amnesia and other cognitive impairments. Bowden (1990) has argued that there is insufficient evidence to conclude that separate neuropathological processes with different site-specific effects are responsible for these two neuropsychological disorders. Bowden noted that most individuals who show the neuropathology characteristic of the Wernicke-Korsakoff syndrome post mortem were not given the clinical diagnosis during their lifetime (Harper et al. 1986; Torvik et al. 1982). Therefore, studies that have examined alcohol-related pathology may have been confounded by the inclusion of undiagnosed cases of alcoholics with Wernicke-Korsakoff syndrome in the non-Korsakoff alcoholic group (see also Dufour, chapter 1).

Since many brain changes may be reversible, length of abstinence also may be a confounding factor in many studies of alcohol-related pathology. Evidence suggests that the findings of structural and functional imaging studies of alcoholics can be influenced by length of abstinence. For example, computerized tomography (CT) studies indicate that cerebral atrophy may be partially reversible with abstinence (Carlen et al. 1978; Ron 1983), and changes in regional and local cerebral blood flow have been observed in alcoholics after a period of abstinence (Berglund and Ingvar 1976; Hata et al. 1987). In studies comparing alcoholics with and without Korsakoff's syndrome, the length of abstinence of Korsakoff patients is generally much longer than that of non-Korsakoff alcoholics.

In this section, studies will be reviewed that show evidence of alcohol-related cerebral pathology in human subjects, particularly in the frontal cortex. Studies demonstrating evidence of damage to structures with connections to the

frontal lobes (e.g., diencephalic structures, basal forebrain nuclei, hippocampus) also will be described. Research findings using animal models of alcohol-related brain damage will be excluded from review, since these studies are covered in other papers of this monograph (e.g., see Walker et al., chapter 11; Hunter, chapter 13).

NEUROPATHOLOGICAL STUDIES

Neuropathological Findings in Non-Korsakoff Alcoholics

Some neuropathological studies of alcoholics suggest that alcoholics have diffuse cortical atrophy. Other studies suggest that cortical atrophy predominantly involves the frontal lobes. For example, Courville (1955) found that alcoholics showed evidence of ventricular enlargement and widespread cortical atrophy that predominantly affected the frontal lobes. Lynch (1960) observed diffuse mild cerebral atrophy and enlargement of the ventricular system in brains of chronic alcoholics. Neubuerger (1957) found mild brain atrophy in less than 50 percent of alcoholics, whereas degeneration of the cerebellar granular layer was present in 67 percent of alcoholics.

Harper and Kril (1989; Kril and Harper 1989) examined the number and pattern of neuronal loss in the brains of male alcoholics (more than half of whom had Wernicke's encephalopathy and/or cirrhosis of the liver) and age-matched nonalcoholic controls ranging in age from 21 to 85. Alcoholics had a significant reduction in the number of large neurons in the superior frontal cortex, and a reduction in the neuronal area of motor, superior frontal, and frontal cingulate gyri. Harper and Corbett (1990) conducted a quantitative study of the basal dendritic arborization of neurons from the superior frontal and motor cortex of alcoholics (a third of whom had Wernicke's encephalopathy or cirrhosis) and age-matched control subjects. Most of these subjects had been included in the neuronal count studies just described. All of the dendritic arborization measurements from the frontal and motor cortex were significantly smaller in alcoholics than in controls. According to the investigators, a retraction of the dendritic arbor may be an important pathogenetic mechanism associated with progressive cognitive impairment in alcoholics. Other findings by Harper and his colleagues are reviewed in chapter 3 of the present monograph.

In a morphological study of four brain regions of nine male chronic alcoholics (four had cirrhosis) and nine controls, Alling and Bostrom (1980) observed changes indicating demyelination of the mammillary bodies in alcoholics. Demyelination was not found in the dorsal and medial part of thalamus, the vermis of the cerebellum, or the quadrigeminal plate. These results suggested that mammillary body damage occurs commonly in alcoholics and is not a phenomenon specific to Korsakoff's syndrome.

According to Freund (1982), molecular changes in the synapses could cause the behavioral deficits associated with alcohol abuse by interfering with

interneuronal communication. Freund and Ballinger hypothesized that chronic alcohol abuse causes destruction of synapses with their receptors, and that impaired synaptic function may exist before gross brain atrophy or end-stage dementia can be demonstrated. In one study, Freund and Ballinger (1988*a*) compared benzodiazepine receptor binding in histologically normal frontal cortices of alcoholics and age-matched controls who were free of other brain or liver diseases. The two groups did not differ in the degree of left ventricle dilatation or frontal cortical atrophy, but alcoholics showed a 20-percent decrease in benzodiazapine receptor binding in the frontal cortex in comparison to controls. In another study, Freund and Ballinger (1988*b*) found no significant differences between alcoholics and controls in brain weights, cortical atrophy, or dilatation of the lateral ventricles. However, in comparison to controls, alcoholics showed a significant decrease of cholinergic muscarinic receptor densities in the frontal cortex. The two groups did not differ in receptor affinities in the frontal cortex. Freund and Ballinger concluded that alcohol may have neurotoxic effects in at least one brain region, the frontal cortex; decreased interneuronal communication associated with the loss of synaptic receptors could cause varying degrees of impaired memory and learning.

This body of data is important in that it suggests significant brain changes in non-Korsakoff alcoholics. Specifically, this evidence implicates a variety of brain areas, including cortex, diencephalon, and cerebellum, and alteration of neuronal structure and/or function, particularly in frontal cortex.

Neuropathological Findings in Korsakoff Patients

As early as 1910, neuropathologists have been aware of cerebral atrophy in alcoholics (Alexander 1945; Mott 1910; Neubuerger 1957). By 1965, a majority of researchers believed that a lesion within the diencephalon was responsible for the signs characteristic of Korsakoff's syndrome, although a few believed that both cortical and diencephalic lesions were associated with this syndrome (Barbizet 1970; Brion 1969; Talland 1965). Both views remain viable today.

Victor et al. (1971) observed mild cortical atrophy primarily involving the frontal regions in 27 percent of cases with Korsakoff's syndrome. Histopathological changes were most frequently found in the thalamus, hypothalamus, and brain stem. The medial dorsal thalamic nucleus was involved in 88 percent of the cases, and the medial mammillary nucleus was involved in 100 percent of the cases.

Kril and Harper (1989) noted that alcoholics with Wernicke's encephalopathy had fewer neurons and a smaller mean neuronal area in the superior frontal cortex than nonalcoholic control subjects. In addition, they had significantly less white matter glia in the frontal cingulate gyrus than controls. This pattern of neuronal loss and shrinkage was similar to that shown by their entire alcoholic group. Victor et al. (1971) found similar pathological changes in cases of Wernicke's dis-

ease with and without Korsakoff's psychosis. According to Harper and Kril (1990), researchers have not yet identified the pathological substrate responsible for the clinical differences in Wernicke's encephalopathy and Korsakoff's psychosis.

Arendt et al. (1983) found that Korsakoff patients had significantly fewer cholinergic neurons of the nucleus basalis of Meynert than nonalcoholic control subjects. In addition, the number of neurons in the external segment of the globus pallidus did not differ in Korsakoff patients and controls. The authors hypothesized that since the nucleus basalis of Meynert is the major source of cholinergic innervation of the cerebral cortex, the selective loss of neurons in this region of the basal forebrain may be responsible for the progressive deterioration of memory and cognitive functions in Korsakoff patients (see also Arendt, chapter 22).

In a neuropathological study of two patients with Korsakoff's syndrome, Mayes et al. (1988) found that both patients showed severe neuronal loss from the medial mammillary bodies and a narrow band of gliosis in the medial thalamus, adjacent to the wall of the third ventricle. One patient showed visible signs of focal cortical atrophy in the frontal and parietal regions; morphometric measures indicated that this patient showed reduced nucleolar volumes and neuronal loss in layers III and V of the frontal cortex and significant neuronal loss in the hippocampus. Although the second patient did not show visible signs of cortical atrophy, he showed reduced nucleolar volumes in layers III and V of the frontal cortex. Both patients showed reductions in nucleolar volume in the hippocampus, septum, locus coeruleus, and the paraventricular and supraoptic nuclei of the hypothalamus. Neither patient showed neuronal loss nor reduced nucleolar volume in the nucleus basalis of Meynert. Both patients had anterograde and retrograde amnesia, but the second patient's performance on anterograde tests was worse than the other patient's. Both patients performed poorly on a test of flexible thinking, the Wisconsin Card Sorting Test (Berg 1948; Lezak 1983), but only the first patient had a deficit large enough to be classified as "frontal"; he also showed more severe perseverative difficulties on the Wisconsin Card Sorting Test and on a verbal fluency test. Mayes et al. speculated that the impairment shown by both patients on a short-term memory task could be caused by frontal lobe atrophy. According to the authors, the findings indicate that frontal and parietal lobe, hippocampal, dorsomedial thalamic, and cholinergic basal forebrain pathologies are variable features of Korsakoff's syndrome.

CT STUDIES

CT Findings in Non-Korsakoff Alcoholics

Reports of abnormalities in CT scans of alcoholics have been variable (see Pfefferbaum and Rosenbloom, chapter 4). In two studies (Fox et al. 1976; Epstein et al. 1977), ventricular enlargement or cerebral atrophy was noted in over half the alcoholic patients studied. In another study (Hill and Mikhael 1979), only one

of 15 chronic alcoholics showed brain abnormalities on CT scans, despite prevalent neuropsychological impairments consistent with frontal involvement in the group as a whole. Shimamura et al. (1988) found that CT scans of alcoholics detected cortical atrophy, as indicated by significantly greater fluid volume than control subjects in the left frontal sulci and the left Sylvian fissure. Cala (1987) compared the CT scans of age-matched alcoholics and nonalcoholic control subjects. For every 5-year age group comparison, the mean atrophy grade of alcoholics was significantly greater than for controls. Furthermore, the frontal lobes were most severely affected in the alcoholics, followed by the temporal, parietal, and occipital lobes experiencing less atrophy.

Gurling et al. (1986) compared CT scans of monozygotic twins who were discordant for alcohol consumption. Members of the twin pairs who were severely dependent on alcohol showed significantly greater frontal brain densities than their normal-drinking cotwins. This effect of increased frontal brain densities in alcoholic twins remained when the effects of cranial size and age were statistically controlled. Investigators hypothesized that the increased brain density occurred because "dendrites and intercellular components have been affected, allowing closer packing of neuronal cell bodies" (p. 767). They also noted that other pathologic mechanisms involving myelin must be involved, since the frontal lobes consist predominantly of white matter.

Mutzell and Tibblin (1989) obtained CT scans of a random sample of 195 men aged 20 to 65 from the general population in Sweden. The subjects were told to abstain from drinking alcohol for 10 hours before the CT scan. The men were divided into low-, intermediate-, and heavy-drinking groups based on self-reports of the amount of drinking during the past week and the presence or absence of three symptoms related to heavy drinking (loss of control over drinking, morning drinks, and blackouts). The results suggested that the frequency of frontal lobe atrophy was significantly higher in the intermediate- and heavy-drinking groups than in the low alcohol consumption group. Unfortunately, no exclusion criteria were used in this study. Therefore, the frontal lobe atrophy observed in some subjects could have been caused by factors other than alcohol consumption, such as head injuries, neurological disorders, or polydrug abuse.

Lishman et al. (1987) reported that a group of alcoholics who had been abstinent for 6 months to 3 years, had significantly greater white matter CT absorption densities in the right frontal region than normal controls. On the other hand, a group of alcoholics abstinent for a longer period (mean of 7 years) had significantly greater white matter absorption densities in the left frontal region than controls. Alcoholics showed significant negative correlations between verbal fluency scores and absorption density measurements in frontal white matter bilaterally. However, they showed significant positive correlations between verbal memory scores and density in measurements of medial thalamic regions bilaterally. Lishman et al.

speculated that "the frontal white matter changes, which rise in relation to deficits, may reflect alcohol neurotoxicity, while the thalamic densities which fall in relation to memory impairments may be attributable to thiamine deficiency" (p. 14).

CT Findings in Korsakoff Patients

Shimamura et al. (1988) found that Korsakoff patients had significantly lower CT density values bilaterally in the region of the thalamus than nonalcoholic control subjects. Also, the right thalamic values of Korsakoff patients were significantly lower than those of non-Korsakoff alcoholics. The right caudate nucleus density values of the Korsakoff patients were significantly lower than those of non-Korsakoff alcoholics and nonalcoholic controls. Korsakoff patients showed greater cortical atrophy than control subjects, indicating greater fluid volumes than controls in the left frontal sulci and in the Sylvian fissures bilaterally. Korsakoff alcoholics differed from non-Korsakoff alcoholics on the left Sylvian fissure fluid measure. Non-Korsakoff alcoholics also showed cortical atrophy, as evidenced by significantly greater fluid volume than control subjects in the left frontal sulci and in the left Sylvian fissure.

When Korsakoff patients were ranked according to their combined scores on 18 cognitive tests, significant correlations were found between test performance and the thalamic CT density and frontal sulcal fluid measures. These findings were interpreted as suggesting that (1) both diencephalic and cortical damage occur in Korsakoff patients, whereas only cortical atrophy occurs in chronic alcoholics, and (2) damage in the thalamic and frontal sulcal regions may strongly contribute to the cognitive and memory impairment associated with Korsakoff's syndrome. The investigators stated that their findings are consistent with the hypothesized link between nutritional deficiency and diencephalic damage and the hypothesized link between alcohol neurotoxicity and cortical atrophy.

Jacobson and Lishman (1990) reported further evidence that Korsakoff patients have both diencephalic and cortical lesions. CT scans indicated that Korsakoff alcoholics had significantly greater mean lateral ventricle brain ratios and third ventricular indices than non-Korsakoff alcoholics and nonalcoholic controls. Sulcal width was significantly greater in Korsakoff patients than in controls. The interhemispheric fissure width of Korsakoff alcoholics was significantly greater than that of controls and non-Korsakoff alcoholics. According to Jacobson and Lishman, widening of the interhemispheric fissure suggests shrinkage in the frontal brain regions. These researchers stressed the idea that diencephalic damage in Korsakoff patients is caused by thiamine deficiency, whereas frontal shrinkage is caused by alcohol neurotoxicity or by other conditions associated with alcoholism (e.g., head trauma, cirrhosis, genetic factors, malnutrition).

MRI STUDIES

MRI Findings in Non-Korsakoff Alcoholics

Jernigan et al. (1991*b*) reported that non-Korsakoff alcoholics showed significantly

greater ventricular and cortical sulcal cerebrospinal fluid (CSF) and a significantly lower diencephalic gray matter volume than nonalcoholic control subjects (see also Pfefferbaum and Rosenbloom, chapter 4; Pfefferbaum and Rosenbloom 1990). No differences in global cortical gray matter volume were found between Korsakoff and non-Korsakoff alcoholics and control subjects. However, post-hoc comparisons of measures of gray matter volume in various cortical regions indicated that relative to control subjects, non-Korsakoff alcoholics showed reductions in parietal and superior frontal regions.

In another study that examined MRIs of a larger sample of non-Korsakoff alcoholics 4 to 5 weeks after detoxification, Jernigan et al. (1991*a*) found that alcoholics had significantly greater ventricular and cortical sulcal CSF and a significantly smaller overall volume of subcortical and cortical gray matter than nonalcoholic control subjects. Alcoholics showed a significantly smaller gray matter volume than controls in all of the subcortical structural measures (caudate, lenticular, and diencephalic) and in three of the cortical measures (dorsolateral frontal, parieto-occipital, and mesial temporal). The investigators noted that the reduced volume of mesial temporal gray matter is consistent with neuropathological reports of cell loss in limbic structures.

In alcoholics, significant correlations were found as follows: (1) between the ventricular CSF measure and each of the subcortical gray matter volume measures and the parieto-occipital gray matter measure, and (2) between the cortical CSF measure and the dorsolateral frontal and parieto-occipital gray matter measures. According to Jernigan and her colleagues, the fact that many significant correlations were found between CSF and gray matter volumes suggests that these measures at least in part reflect neuropathological brain changes in alcoholics.

Although several significant negative correlations were found between CSF volume measures and performance on cognitive tests, there was little evidence of relationships between gray matter measures and cognitive test scores. The authors stated that the cortical and subcortical volume changes found in alcoholics could be caused by the neurotoxic effects of ethanol or by nutritional deficiencies.

Chick et al. (1989) found significant correlations in alcoholics between MRI T1 values in frontal white matter and degree of impairment on the Maudsley Category Sorting Test as assessed by four different measures: categories, sorts, errors, and perseverations. Alcoholics also showed significant correlations between the following variables: (1) T1 values in parietal white matter and scores on categories, errors, and perseverations; (2) T1 values in frontal gray matter and categories scores; and (3) whole-brain T1 values and all four category test measures. Alcoholics had significantly higher MRI T1 values than nonalcoholic controls in the frontal gray and parietal white matter regions as well as in the whole-brain measure.

MRI Findings in Korsakoff Patients

In an MRI study, Jernigan et al. (1991*b*) found that Korsakoff alcoholics had sig-

nificantly greater ventricular and cortical sulcal CSF and a significantly lower diencephalic gray matter volume than nonalcoholic control subjects. The Korsakoff alcoholics differed from the non-Korsakoff alcoholics in several ways: greater ventricular CSF, lower gray matter volume in the anterior diencephalon (i.e., septal nuclei and anterior hypothalamic gray), and smaller gray matter volumes in mesial temporal and orbitofrontal cortex. The pattern of cortical and subcortical changes shown by Korsakoff alcoholics in this study was similar in many ways to non-Korsakoff alcoholics in the Jernigan et al. (1991*a*) study. Jernigan et al. speculated that "some acute event, resulting in damage to specific cortical–subcortical structures, may be responsible for the neurological and neuropsychological differences between these two groups" (p. 425).

Christie et al. (1988) found that Korsakoff patients had significantly higher MRI T1 values than nonalcoholic control subjects in the following regions: frontal gray matter right and left, frontal white matter left, parietal gray matter left, and parietal white matter right. In comparison to controls, the Korsakoff patients showed significant cortical atrophy. In Korsakoff patients, there was a significant negative correlation between atrophy scores on MRI and performance on only one of several neuropsychological tests administered. Christie et al. interpreted the increased T1 values in gray and white matter in frontal and parietal cortex in Korsakoff patients as reflecting neuronal degeneration.

FUNCTIONAL BRAIN CHANGE STUDIES

Functional Brain Changes in Non-Korsakoff Alcoholics

Melgaard et al. (1990) measured regional cerebral blood flow (rCBF) by single photon emission computerized tomography (SPECT) of inhaled Xe-133 in alcoholic and nonalcoholic males. Compared to control subjects, the alcoholics showed significant flow reduction in the anteromesial frontal region, in the mesial part of the right occipital lobe, and in a small area in the left parietal region.

When alcoholics were divided into two groups based on the severity of their alcohol abuse, the more severe alcoholics showed a greater flow reduction in frontal cortical and periventricular regions. When the alcoholics were divided into two groups based on the severity of their intellectual impairment, the more impaired group showed a greater flow reduction in frontal cortical and periventricular regions as well as in regions around the Sylvian fissures.

Dally et al. (1988) measured rCBF in young alcoholics and control subjects. These investigators found that all except two of the alcoholics showed a hypofrontal flow distribution. In contrast, 75 percent of the controls had a hyperfrontal flow distribution. The alcoholics exhibited reduced overall hemispheric gray matter blood flow bilaterally in comparison to the controls. Berglund and Ingvar (1976) compared rCBF in older and younger alcoholics and found that older alcoholics

showed a reduction in mean flow in the lower frontal region of the brain and in the anterior temporal region in comparison to the younger alcoholics.

Berglund et al. (1987) measured rCBF in alcoholics after 1, 3, 5, and 7 weeks of abstinence. During each measurement session, alcoholics had significantly lower mean rCBF levels than control subjects. In the older alcoholics, the mean rCBF level increased significantly from the first to the seventh week of abstinence. In comparison to the controls, alcoholics had a significantly lower rCBF distribution in the right frontal lobe after 1 and 7 weeks of abstinence. After 1 week of abstinence, the degree of hemispheric flow asymmetry (right minus left) shown by older alcoholics was significantly smaller than that of older control subjects in two superior frontal and two inferior temporal regions. After 7 weeks of abstinence, the degree of asymmetry in alcoholics was smaller in only one superior frontal region.

Samson et al. (1986) studied regional cerebral glucose utilization in chronic alcoholics. Compared to age-matched control subjects, alcoholics showed a significant decrease in the regional distribution index in the upper medial frontal region, suggesting selective hypometabolism in this region. Gilman et al. (1990) obtained similar results using the positron emission tomography (PET) technique: Alcoholics with and without alcoholic cerebellar degeneration showed hypometabolism in the medial frontal region of the cerebral cortex. In the combined group of alcoholics with and without cerebellar degeneration, significant correlations were found (1) between frontal lobe metabolism and errors on the Category Test of the Halstead neuropsychological battery (see Lezak 1983), and (2) between local cerebral metabolic rate for glucose normalized to the cerebral cortex and CT atrophy for the rostral medial frontal cortex bilaterally. Gilman et al. (1990) suggested that the latter correlation may indicate that the frontal lobe hypometabolism may reflect loss of tissue. If this were the case, however, their data could not differentiate "whether the residual tissue is hypometabolic or is normally active metabolically" (p. 783).[5]

Porjesz et al. (1987) used the event-related potential (ERP) technique to assess the P3 component in alcoholics to motivationally significant stimuli. Alcoholics and a control group of nonalcoholics were required to respond to equiprobable task-relevant visual stimuli (the number 0.00 or 1.00) in a baseline condition and in two incentive conditions. In all experimental conditions, P3 amplitude was significantly lower in alcoholics than in controls. In the control group, P3 amplitude increased to the high-incentive stimuli but not to the low-incentive stimuli. Alcoholics, on the other hand, did not show a differential P3 response to the high-incentive stimuli. The investigators interpreted their finding to support the view that alcoholics have frontal lobe and/or limbic system

[5] *Martin et al. (1992) made similar observations on patients with alcoholic organic mental disorders.*

deficits. According to Porjesz et al., the results, together with their previous finding that alcoholics maintained low amplitude P3s both to rarely occurring target stimuli and to frequently occurring nontarget stimuli (Porjesz and Begleiter 1982), indicated that alcoholics have multiple brain deficits, especially in frontal and medial temporal lobe regions. For further discussion on brain electrophysiology, see Porjesz and Begleiter, chapter 5.

Functional Brain Changes in Korsakoff Patients

Hunter et al. (1989) examined the pattern of rCBF in Korsakoff patients using SPECT. In comparison to nonalcoholic control subjects, Korsakoff patients showed a trend toward reduced blood flow in frontal areas. The Korsakoff patients showed several significant correlations between the degree of reduction in rCBF in frontal areas and the degree of impairment on memory and orientation tests. Like Gilman et al. (1990), Hunter (1990) noted that frontal metabolic deficits "could mean that a normal tissue mass has reduced neuronal activity, or that a reduced tissue mass has normal levels of activity or some mixture of the two" (p. 455). Hunter further noted that since some CT and neuropathological studies point to structural loss of gray and white matter in the frontal lobes of Korsakoff patients, the frontal metabolic impairment in this region probably at least in part reflects reduced tissue mass.

Hata et al. (1987) obtained three-dimensional measurements of local cerebral blood flow (lCBF) in Korsakoff and non-Korsakoff alcoholics using stable xenon inhalation as a contrast medium for CT. Measurements were obtained before and after treatment (2 to 28 weeks after treatment in Korsakoff patients, and 2 to 6 weeks after treatment in non-Korsakoff alcoholics). Before treatment, the non-Korsakoff alcoholics showed symptoms and signs of neurotoxicity without fixed neurological signs. In comparison with nonalcoholic control subjects, the Korsakoff and non-Korsakoff alcoholics showed significant reductions in lCBF values for gray matter in the following regions: frontal cortex, temporal cortex, occipital cortex, cingulate gyrus, caudate nucleus, putamen, and thalamus. In comparison with non-Korsakoff alcoholics, the Korsakoff patients showed significant reductions in lCBF values for gray matter in the cingulate gyrus, hippocampal gyrus, hypothalamus, and nucleus basalis of Meynert. Korsakoff patients showed a significant reduction in lCBF in frontal white matter compared with non-Korsakoff alcoholics and nonalcoholic controls. After treatment, Korsakoff as well as non-Korsakoff alcoholics showed diffuse increases of lCBF throughout the brain, which correlated with improvements in cognitive performance. Hata et al. hypothesized that alcohol has neurotoxic effects on the nucleus basalis of Meynert and adjacent systems, and that "damage to these basal forebrain nuclei would impair their cholinergic projections resulting in diffuse reductions in cortical and subcortical blood flow" (p. 43).

CONCLUSIONS AND SUMMARY OF EMPIRICAL DATA

In summary, there is considerable neuropathological, neuroradiological, and functional imaging evidence in favor of the view that alcohol abuse can lead to diffuse brain damage involving cortical as well as subcortical brain regions. Studies have not consistently demonstrated different patterns of cortical and subcortical damage in Korsakoff and non-Korsakoff alcoholics. Many studies suggest that frontal systems of the brain are affected by alcohol abuse, particularly in the Korsakoff alcoholic.

In human studies of alcoholism, it is difficult to determine whether brain changes precede or follow alcohol abuse, or whether the brain changes could be associated with problems that frequently accompany alcoholism (e.g., malnutrition, head injury, liver disease, abuse of other drugs). Direct associations between alcohol toxicity and brain changes would be supported by two types of findings: (1) a partial reversal of atrophy and functional impairment following abstinence from alcohol and (2) the presence of significant correlations between amount of brain atrophy and quantity of drinking (Wilkinson 1982). Since neither of these associations has been definitively established in the studies reviewed, the question remains ripe for future research.

NEUROBEHAVIORAL OBSERVATIONS CONSISTENT WITH FRONTAL SYSTEM INVOLVEMENT AFTER CHRONIC ALCOHOL INGESTION

Certain neuropsychological vulnerabilities are more likely observed in alcoholic Korsakoff patients than in non-Korsakoff alcoholics. Other neuropsychological abnormalities may overlap considerably between the two groups. Dysfunctions of notable overlap include reduced attentional and visuospatial abilities that rely heavily upon cortical sensory regions and association areas. By contrast, Korsakoff patients have shown significantly diminished motivational and emotional abilities, as well as confabulation about life events. However, non-Korsakoff alcoholics' deficits have been mild or nonexistent. The emotional, motivational, and confabulatory abnormalities in Korsakoff patients have been tied to pathology in frontal–diencephalic, limbic, and basal–forebrain structures. Moreover, these damaged brain systems may be directly involved in the anterograde amnesia of alcoholic Korsakoff's syndrome (Jernigan et al. 1991*a*,*b*; Kopelman 1991; Oscar-Berman 1992).

Support for the view that chronic ethanol intake has deleterious effects on prefrontal cortical functioning requires the demonstration of frontal-like deficits

in alcoholics. As Walsh (1987) points out, however, many frontal-like symptoms, especially in their subtle form, escape detection with standardized neuropsychological tests yielding quantitative data. In addition, since there are no definitive tests pathognomonic of frontal dysfunction alone, it is necessary to rely upon a range of tests. Poor performance on several tests on which patients with focal prefrontal lesions show deficits implies a high likelihood that the deficits indeed are associated with frontal damage. As Walsh (1991) states, "After all, it is possible to diagnose localized frontal pathology reliably with neuropsychological examination even though some elements (test performances) may not be present in the individual case" (p. 51).

To assert with confidence that frontal lobe damage specifically is responsible for the neurobehavioral deficits, however, requires demonstrating first that the deficits do not reflect decline from diffuse damage throughout the brain, causing impairments in processes essential for cognitive functioning in general. To establish the extent to which frontal-lobe deficits contribute to memory loss, frontal deficits in Korsakoff patients would have to be dissociated from impairments in non-Korsakoff amnesic patients whose memory loss derives from a different etiology (without direct frontal involvement, for example, anterior communicating artery disease). Further, it would be helpful to demonstrate that alcoholics show the same neurobehavioral impairments as patients with circumscribed damage to prefrontal cortex.

To date, not all frontal-like characteristics have been found in alcoholics. As Walsh (1991) points out, however, this should not be surprising "since not all patients with proven frontal pathology have all the symptoms and signs...of the frontal lobe syndrome" (p. 51). In discussing alcoholism without amnesia, Walsh continues, "There is evidence that there may be sub-syndromes related to different areas of the frontal lobes (dorsolateral, basal and medial. . .). If this is so, then it may well be that with greater accent on dorsolateral atrophy shown in neuropathological studies, corresponding differences of intellectual regulation may be more commonly associated with alcohol related brain damage than, say, difficulties of self motivation or the flexible control of excitation/inhibition which are more characteristic of damage to the medial and basal areas respectively" (the latter being important in Korsakoff's syndrome).

Apparently, not only does frontal dysfunction occur with chronic alcohol abuse, but the two frontal subsystems, dorsolateral and ventral/orbitofrontal, are differentially affected in Korsakoff and non-Korsakoff alcoholics (Harper and Kril 1989; 1990; Jernigan et al. 1991*a*,*b*; Oscar-Berman et al. 1991, 1992; Walsh 1991). In this section, evidence will be reviewed indicating that alcoholic Korsakoff patients show signs of damage to both frontal subsystems. That is, they have cognitive deficits (e.g., in problem-solving, concept learning, response shifting, organizing new information, and performing delayed-reaction tasks related to dorsolateral prefrontal function) as well

as emotional abnormalities (e.g., in perceiving emotional stimuli, overactivity, and disinhibition associated with ventral/orbitofrontal function). Alcoholics without Korsakoff's syndrome show milder deficits than Korsakoff patients. Their deficits seem to reflect dorsolateral prefrontal damage rather than damage in the ventral orbitofrontal system (Oscar-Berman 1992; Oscar-Berman et al. 1990).

EMPIRICAL INVESTIGATIONS

Early Studies

Frontal lobe dysfunction has been implicated in alcohol-related brain damage since Korsakoff described frontal-like symptoms in patients with the syndrome bearing his name. Such patients perseverate, that is, they frequently repeat certain phrases or actions (Victor and Yakovlev 1955). Meissner (1968) and Talland (1965) described similar behaviors in alcoholic Korsakoff patients, as well as personality changes reminiscent of patients with bilateral frontal lobe damage, giving the impression of apathy and indifference (see also Oscar-Berman 1992). Thus, early research on chronic alcoholics provided evidence for the role of the frontal lobes in Korsakoff's syndrome. Despite this early work and accumulating neurobehavioral and neuropathological evidence (for reviews, see Bowden 1990; Harper and Kril 1989, 1990; Oscar-Berman et al. 1991), there remains considerable controversy regarding the role of the frontal lobes in alcohol-related cognitive dysfunction.

In our laboratory, we explored the nature of alcohol-related neurobehavioral changes by adapting sensitive experimental paradigms from physiological psychology for use with clinical neurological populations (Oscar-Berman 1984). Initially, we assessed strategy formation and its possible disruption in brain-damaged groups (Oscar-Berman 1973). We observed striking deficits in patients with Korsakoff's syndrome. The deficits included abnormal perseverative responding reminiscent of monkeys with prefrontal lesions (Oscar and Wilson 1966). In subsequent studies, other methodologies confirmed the presence of strong response perseveration in Korsakoff patients (Butters and Cermak 1980; Moscovitch 1982; Oscar-Berman 1984; Tarter and Parsons 1971).

Later Studies

In the 1970's, research on the neuropsychological consequences of alcoholism began to flourish. Several investigators noted neuropsychological abnormalities in Korsakoff patients compatible with clinical signs of frontal lobe pathology (Lezak 1983; Lhermitte et al. 1986; Lhermitte and Signoret 1976; Luria 1980). For example, patients with Korsakoff's syndrome performed poorly on tasks of divided attention (Glosser et al. 1976; Talland 1965) and selective attention (Oscar-Berman and Samuels 1977); they showed evidence of hypoarousal on tests of autonomic arousal and habituation (Oscar-Berman and Gade 1979); and they were highly susceptible to interference (Butters and Cermak 1980; Moscovitch 1982).

Sullivan et al. (1993) tested non-Korsakoff alcoholics, schizophrenics, uni-

lateral frontal-lobe patients, and normal control subjects on the Wisconsin Card Sorting Test (Heaton 1981). Errors were subjected to a principal components statistical analysis to determine the factors contributing to poor performance; analysis revealed perseveration, inefficient sorting, and nonperseverative errors. Although the alcoholic and schizophrenic groups were impaired on conventional scores of the test, only the schizophrenics and the frontal patients made significant errors of perseveration. The authors concluded that the validity of the Wisconsin Card Sorting Test as an index of dorsolateral prefrontal cortical function depends upon the "factor analyzed and the group considered" (p. 24). Anderson et al. (1991) made similar observations on the Wisconsin Card Sorting Test.

Other investigators have demonstrated frontal-like neuropsychological features in Korsakoff patients. For example, Shimamura et al. (1991) compared Korsakoff and non-Korsakoff amnesics with frontal lobe patients on the Initiation-Perseveration Index of the Dementia Rating Scale (Mattis 1976). They found that the Korsakoff patients were as impaired as the frontal patients. Furthermore, the Korsakoff and non-Korsakoff amnesic patients were impaired on the Memory Index of the same test, whereas the frontal patients were not. This study confirms other findings that abnormal perseverative responding is dissociable from poor performance on certain tests of memory function, although both deficits are characteristic of patients with Korsakoff's syndrome (e.g., Freedman and Oscar-Berman 1986*a*; Moscovitch 1982).

In a similar vein, Joyce and Robbins (1991) administered traditional neuropsychological tests of frontal function and tests of planning and spatial memory to Korsakoff and non-Korsakoff alcoholics. Consistent with the dissociation between memory deficits and frontal-like characteristics already mentioned (e.g., Freedman and Oscar-Berman 1986*a*; Shimamura et al. 1991), the Korsakoff patients demonstrated deficits on the planning task that were not explicable by their abnormalities in memory or visuoperceptual functions. Rather, the deficits were attributed to poor strategy organization and abnormal perseveration characteristic of frontal damage (see also Oscar-Berman 1973). Compared to normal controls, the non-Korsakoff alcoholics demonstrated mild deficits on the frontal tests. The pattern of deficits was not indicative, however, of specific frontal dysfunction. In contrast to the results of Joyce and Robbins (1991), Wiegersma et al. (1991) found no evidence of frontal deficits in Korsakoff patients on subjective ordering tasks.

As noted earlier in this chapter, there are many methodological differences among studies contributing to the lack of agreement in results obtained from different laboratories. Another variable that may affect outcome is family history of alcoholism. Peterson et al. (1992) administered a battery of neuropsychological tests to nonalcoholic sons of male alcoholics and to nonalcoholic controls with no history of familial alcoholism. Half the

subjects in each group were tested while sober, and half were tested while alcohol intoxicated. Alcohol-related decrements in functions associated with prefrontal cortex (the organization of novel information) were apparent in the familial group only, although both groups showed deficits on memory tasks associated with temporal cortical involvement.

Comparative Neuropsychology and Frontal Dysfunction

The contribution of frontal dysfunction to alcohol-related cognitive impairments was assessed using the comparative neuropsychology approach (Freedman and Oscar-Berman 1986*b*; Oscar-Berman 1984). That is, we applied tasks known to be reliable and valid measures of brain function in nonhuman primates to elucidate the nature of the deficits in human neurological groups. In three studies, we employed delayed-reaction tasks such as DR and DA (Freedman and Oscar-Berman 1986*a*; Oscar-Berman et al. 1982, 1991). In all three studies, these experimental paradigms were selected because of their special sensitivity to frontal-lobe damage. We specifically included alcoholics with and without clinical signs of Korsakoff's syndrome to determine whether the groups would differ in their performance profiles.

In our initial study (Oscar-Berman et al. 1982) we reported clear deficits by alcoholic Korsakoff patients on classical DR and DA tasks with monetary rewards for correct responding. In a followup study (Freedman and Oscar-Berman 1986*a*), we used the same methods and procedures. This time we studied DR and DA performance in Korsakoff patients and in neurological groups chosen specifically for important differences in their neuropsychological characteristics. One group, consisting of patients with CT-documented bilateral frontal lobe lesions, was included to establish baseline levels attributable to known prefrontal pathology. Another group consisted of patients with amnesia from anterior communicating artery disease (ACoA) to assess the contribution of amnesia unrelated to alcoholism.

Our results supported the claims of Pribram and his colleagues that bilateral frontal lobotomies lead to DA deficits (Pribram et al. 1962). Our frontal patients had the most severe deficits on DA and DR tasks, but the Korsakoff patients also were impaired. Deficits on delayed-reaction tasks correlated with degree of perseverative responding on the Wisconsin Card Sorting Test (Heaton 1981; Lezak 1983) but did not correlate with short-term memory loss as measured by the Wechsler Memory Scale (Wechsler 1945). This pattern of results strongly suggested that perseveration is dissociable from memory factors and that deficits by Korsakoff patients on DA and DR were due to brain damage separable from that causing amnesia.

Another study of performance on DR tasks was designed to measure neuropsychological deficits in addition to those linked directly with prefrontal damage. The experimental design was somewhat unique in being able to evaluate several functions simultaneously: perceptual processing in two different sensory

modalities, short-term memory, and spatial memory (Oscar-Berman et al. 1992). The experiment was modeled after one used to measure similar functions in monkeys with frontal lesions (Oscar-Berman 1975, 1978). An indirect DR procedure was administered with an automated test apparatus. Colors or tones served as the stimuli, and pennies were the reinforcers (although subjects actually earned 5 cents for each penny). Figure 4 depicts the apparatus, which contained three small speakers about 6 in apart, and below the speakers, three small light panels. Sounds came from the speakers, and colors were displayed on the light panels. Response bars were below the left and right lights, and a coin receptacle sat in the middle between the two response bars. The stimuli were red and green lights for the visual tasks or high- and low-pitched tones for the auditory tasks. Each subject was given four DR tasks, two with visual stimuli (spatial or nonspatial) and two with auditory stimuli (spatial or nonspatial). In the visual–spatial DR task, the color of a stimulus and/or its left/right position could be used to solve the problems. For any given subject, one light (red or green) was always presented on the left side, and the other color was always on the right side. Correct responses required

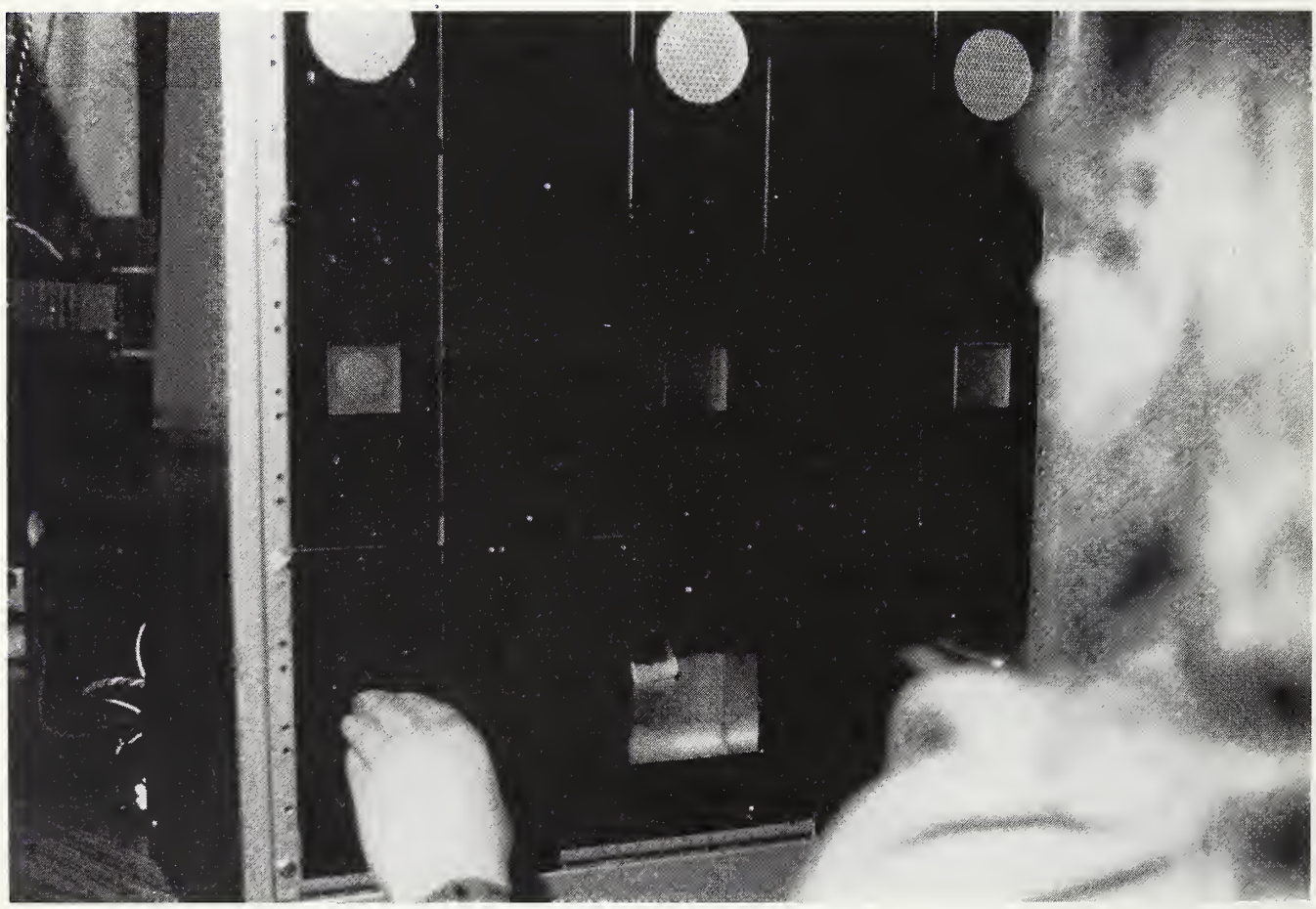

FIGURE 4

The apparatus used to measure performance on visual and auditory, spatial and nonspatial delayed-response tasks.

the subjects to press the lever located directly below the stimulus.

The visual–nonspatial task differed from the spatial task in only one way: The stimuli were presented centrally rather than left and right. In other words, only color, and not spatial location, could be used to solve the problem. The subjects who had received red stimuli on the left side and green stimuli on the right side continued to be reinforced for pressing the left lever when red appeared centrally and for pressing the right lever when green appeared centrally. This condition required that subjects solve the problems from the color cues only, with the spatial cue being irrelevant. We reasoned that if spatial memory was important to any group, removing the spatial cue would hurt their performance.

The procedures for the auditory–spatial and auditory–nonspatial tasks were identical to those described for the visual tasks; the only difference was the sensory modality. Two tones of different frequencies were used rather than two different colored lights. In the auditory–spatial task, the frequency of a tone or its spatial location could be used to solve the problem: For example, when a high-pitched tone (2,000 Hz) came from the speaker on the right, the subject would press right; when a low tone (300 Hz) came from the speaker on the left, the subject would press left. In the auditory–nonspatial task, all of the tones came from the center speaker, and only the tone's frequency, not its spatial location, could be used to solve the problem (i.e., high tone, press right; low tone, press left).

Whether the task was visual or auditory, the stimuli were first presented without any time constraints to facilitate the subjects learning the tasks: They would press one bar for one sound or color, and the other bar for the other sound or color. Next, a delay was introduced between the offset of the stimulus and the opportunity for the subject to respond. To measure short-term memory, there were four different delay periods, presented in the following order: 0, 5, 15, and 30 seconds. Within each delay condition, processing time also was varied by randomly presenting the stimuli for five different periods of time: 20, 40, 80, 160, and 320 milliseconds. The findings indicated that processing time and short-term memory were important parameters for distinguishing among the groups, whereas spatial memory per se was not.

The groups of subjects consisted of non-Korsakoff alcoholics, alcoholics with Korsakoff's syndrome, and normal controls. Controls and the non-Korsakoff alcoholics were equated for age within two age brackets: young, which we defined as up to age 49, and older, which we defined as 50 and over. Those four subgroups (i.e., young and older alcoholics, and young and older controls) formed the basis for one set of statistics looking at the separate contributions of aging and alcoholism. We were interested in evaluating the premature aging hypothesis, which suggests that alcohol abuse accelerates normal chronological aging (see Ellis and Oscar-Berman 1989; Ryan 1982). We reasoned that if the brain changes associated with normal aging are similar to those induced by ethanol consumption, behavioral paral-

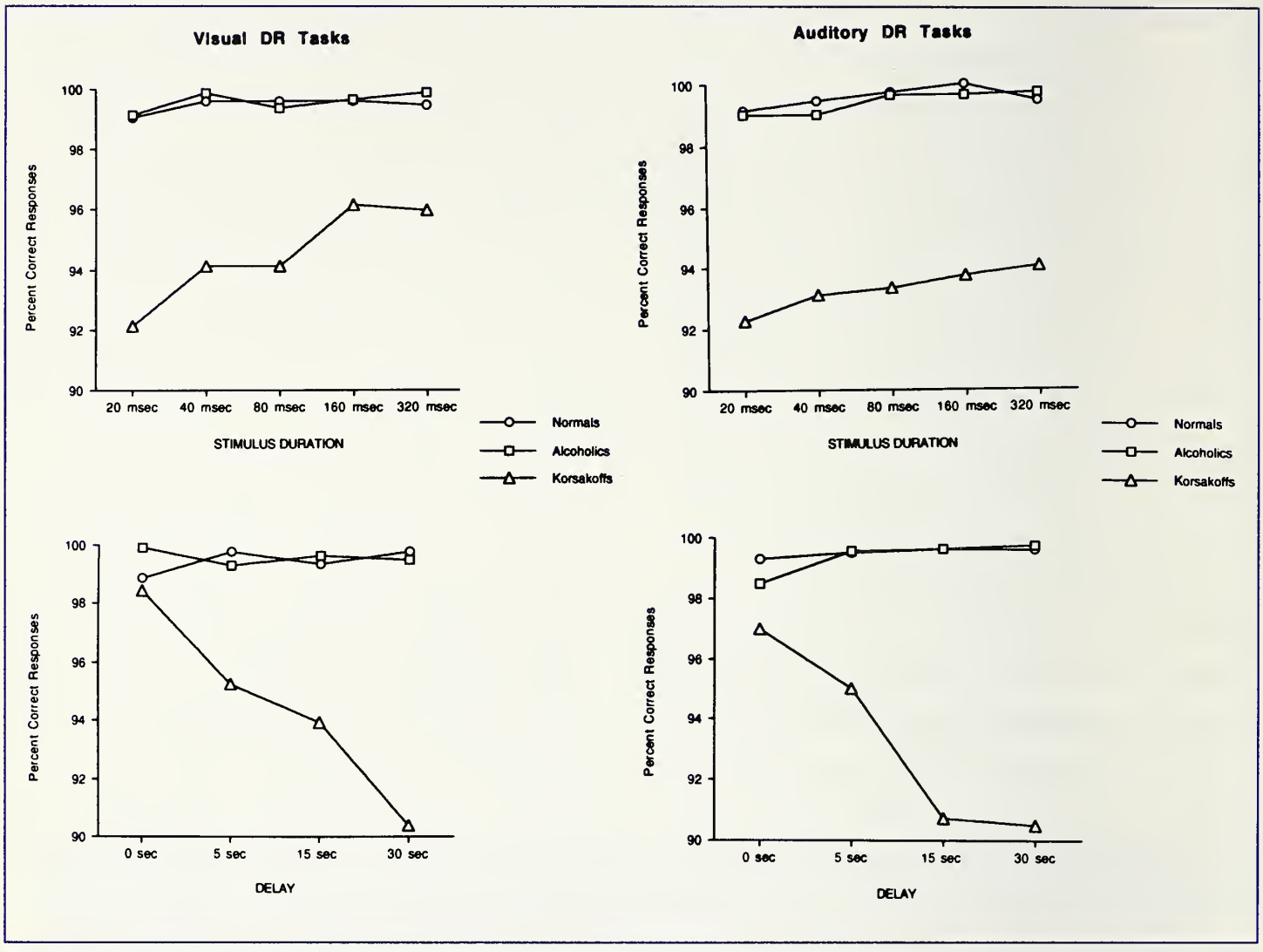

FIGURE 5

Performance of the Korsakoff, alcoholic, and nonalcoholic subgroups equated for age. Reproduced with permission from Oscar-Berman et al. (1992.)

lels should be observed in alcoholics and chronologically older nonalcoholic controls. If the brain changes are different, behavioral manifestations of these differences should be apparent. Since the age range of the Korsakoff patients was between 52 and 68, we did not look for age effects; rather, we compared the Korsakoff subjects with matched non-Korsakoff alcoholics and controls.

Results of the experiment indicated that the groups did not differ in ability to learn the simple task of pressing the left lever for one color or sound and pressing the other lever for a different color or sound. Also, they all benefited equally from having the spatial cue available; in other words, the spatial tasks were easier than the nonspatial tasks. However, there were important group differences. Figure 5 shows comparisons among the age-matched Korsakoff alcoholics, non-Korsakoff alcoholics, and controls. Although the latter two groups did not differ from each other, the Korsakoff individuals were impaired. Whether the DR task was presented in the visual or the auditory modality, the Korsakoff patients were less proficient than their age-matched peers. Impairments by the

Korsakoff patients became exaggerated as demands were placed on short-term memory (long delay intervals, over 15 seconds), and on visual processing time (brief exposures, up to about 80 milliseconds). In other words, not only did the Korsakoff patients display their classic short-term memory deficit, they also did not process incoming information as fast as other people. These results paralleled findings reported in earlier studies of visual processing and memory deficits of Korsakoff patients performing other delay tasks (Oscar-Berman and Bonner 1985, 1989). Further, the results extended the findings to auditory short-term memory impairments in DR tasks, although the Korsakoff subjects were not abnormally hampered by short stimulus duration exposures in the auditory modality.

Interestingly, the Korsakoff patients did not differ from the other groups with respect to their use of spatial cues. As noted earlier, all subjects benefited significantly from the use of spatial cues, and this was especially true when stimuli were brief and visually presented. In an earlier study with monkeys, we found that animals with lesions of orbitofrontal cortex maintained the same normal use of spatial cues in DR tasks as did the Korsakoff subjects. By comparison, however, monkeys with dorsolateral prefrontal lesions did not effectively use the spatial dimension as a cue for successful DR performance (Oscar-Berman 1978). This finding suggests greater involvement of orbitofrontal than dorsolateral prefrontal cortex in Korsakoff's syndrome. It might be noted that such findings are consistent with neuroradiological evidence discussed earlier (Jernigan et al. 1991*a*,*b*).

To determine the extent to which the DR deficits reflected memory loss from nonfrontal damage in our study, we computed Pearson correlation coefficients between scores on DR tasks that had yielded significant group differences and a clinical measure of memory deficit. Correlation coefficients ranged from –0.03 to 0.10 for the Korsakoff patients, –0.34 to 0.20 for the non-Korsakoff alcoholics, and 0.28 to 0.65 for the controls. No correlation was statistically significant for any of the groups. In other words, DR performance was unrelated to memory ability, and the Korsakoffs' memory impairments were unrelated to their DR deficits. Memory loss and frontal lobe deficits appeared to be independent in these patients.

To evaluate the effects of age on DR performance, we also compared the profiles of the four age-matched groups of non-Korsakoff alcoholics and normal controls. We found considerable overlap among the four subgroups. Generally, however, the older subjects (with or without a history of alcoholism) showed deficits in processing and short-term memory, although their deficits were not nearly so dramatic as those of the Korsakoff patients. Older subjects generally performed more poorly than younger subjects under the most difficult conditions. These differences were absent in the easier conditions. Possibly, the older subjects' deficits reflect cortical atrophy characteristic of normal chronological aging (Wilkinson and Carlen 1982). It

should be noted that there were two instances in which the older alcoholics showed the largest deficits of the four groups, but the additive effects of alcoholism and aging were small. Nonetheless, these instances provided some support for a version of the premature aging hypothesis (Ryan 1982) that places emphasis on the appearance of alcohol-related deficits in older subjects. This pattern of results is consistent with findings from other studies of premature aging effects in alcoholics (Ellis and Oscar-Berman 1989; Oscar-Berman and Bonner 1985, 1989; Ryan 1982).

In summary, results of our study of indirect-method DR performance showed that (1) alcoholic Korsakoff subjects, and to a lesser degree, older subjects with or without an alcohol history, were retarded in perceptual processing ability; (2) the Korsakoff patients clearly had short-term memory deficits; (3) use of spatial cues did not differentiate among the groups; (4) older subjects generally performed more poorly than younger subjects under the most difficult conditions but not under the easier conditions; and (5) the combination of alcohol abuse with aging was reflected in minor effects on task performance, thus lending only minimal support to the premature aging hypothesis.

We have studied DR and DA performance in other neurological groups with known or suspected prefrontal cortical damage. Results of those comparisons are described elsewhere (Oscar-Berman et al. 1991). In general, the findings indicated that the patient groups with DR impairments also could be characterized as having severe deficits in visuospatial–mnemonic and attentional functions. Moreover, the patient groups with DA impairments could be characterized as having abnormal perseverative response tendencies. In other words, whereas the presence of anterograde amnesia did not, in itself, present a major obstacle to successful performance on DR, visuospatial abnormalities and attentional deficits may have. Furthermore, intact inhibitory response mechanisms appeared to be needed for successful DA performance.

Another point, regarding the use of language in delayed-reaction tasks, emerged from our studies. In human DR and DA performance, cross-temporal bridging can be mediated by language. The use of language-related strategies, however, is not essential to successful performance on the tasks. This assertion is based mainly upon results with Broca's aphasics, whose expressive language capacity was minimal, but whose DR and DA performance levels were intact (Oscar-Berman et al. 1982). In addition, DR performance was deficient in Parkinson patients, whose language function is mildly impaired (showing anomia; Cummings and Benson 1983), but their DA performance was intact.

Besides DR and DA tasks, several other tasks adapted from animal models have been administered to alcoholics. The results consistently showed that Korsakoff patients were impaired on tasks of frontal function (Oscar-Berman and Zola-Morgan 1980*a*,*b*). For example, on delayed matching-to-sample (DMTS) and delayed non-matching-to-sample

(DNMTS) tasks in which stimulus durations and delays were varied (similar to the indirect-method DR tasks), Korsakoff patients showed a similar decay in performance with increasing delays. They also displayed the same sensitivity to brief visual inputs (Oscar-Berman and Bonner 1985, 1989). Interestingly, Witt and Goldman-Rakic (1983) created a monkey model of Korsakoff's syndrome by mimicking the nutritional deficits of the disease through thiamine deprivation; the monkeys, like Korsakoff patients, were deficient on DNMTS tasks.

SUMMARY AND CONCLUSIONS

The typical symptoms of prefrontal damage in humans are dramatic changes in personality as well as elusive cognitive abnormalities. Personality changes include disinhibition and lack of concern for the consequences of strange behaviors. Intellectual or cognitive changes are mild but unmistakable, most markedly appearing as abnormal perseverative responding. In nonhuman primates, a salient consequence of bilateral frontal lobe damage is failure to perform normally on delayed-reaction tasks. In addition, deficits on delayed-reaction tasks also have been demonstrated in humans with bilateral prefrontal damage. The major neuroanatomical subsystems within the primate frontal lobes are the dorsolateral and polar regions, and the ventral prefrontal region including the orbitofrontal surface and inferior convexity.

There are a few conclusive neuropathological and brain-imaging studies on the relationship between prolonged alcohol consumption and frontal system damage, and there is considerable and growing neurobehavioral evidence of such a link. Clinical and experimental indications of frontal system involvement in neuropsychological dysfunctions of alcoholic patients, especially those with Korsakoff's syndrome, include the following: emotional abnormalities, disinhibition and perseverative responding, reduced problemsolving abilities, poor attention, and deficits on classical tests of bilateral frontal lobe lesions in monkeys (e.g., DR and DA). Some data suggest that the orbitofrontal regions may be differentially involved in Korsakoff patients. It is important, however, to reiterate that differences in neuroanatomy, neurochemistry, and behavioral functions between the two prefrontal systems are relative rather than absolute. Finally, deficits on many tasks are mild (if present) in non-Korsakoff alcoholics.

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MEMORY DEFICITS IN ALCOHOLIC KORSAKOFF PATIENTS

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INTRODUCTION

The purpose of this chapter is to review briefly the study of the underlying cognitive contributors to the memory disorders of alcoholic Korsakoff patients and to give something of the flavor of contemporary research on this topic. Many paradigmatic details of individual experiments have been omitted to focus on the issues of general response patterns as well as theoretical models. First, we explore the learning difficulties experienced by these patients, who have suffered massive brain degeneration, particularly in the area of the diencephalon (Butters and Stuss 1989), and are therefore unable to learn and remember any new information, a phenomenon called "anterograde amnesia."

ANTEROGRADE AMNESIA

The history of the study of memory and memory disorders reflects a push-pull dynamic between two major camps of theoreticians. One camp believes that memory can be captured within a storage system in the brain. This view often proposes that the brain "handles" information by placing it into one of the various types of memories, such as short-term memory or long-term memory. There it resides until some form of retrieval is needed. The alternative view is that memory is not so much a storage bin as it is a form of processing. The form of encoding or processing influences the accessibility of information retrieval. This approach has led to theories that the "depth" of encoding (defined later) determines the probability of retrieval under specific stimulus characteristics (Craik and Lockhart 1972). At times, the systems theories have predominated, at other times, the processing theories.

During the 1960's, systems theories were clearly dominant, and researchers largely concluded that anterograde amnesia in alcoholic Korsakoff amnesics reflected an impaired long-term memory. Most investigators (Baddeley and Warrington 1970) felt that the patients had an intact short-term memory, because they could repeat back short strings of numbers and

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retain material for very brief intervals. The problem for the patient was in long-term memory, where information was obviously not available. Whether this was the consequence of an impaired retrieval mechanism or an inability to initially process the material into a retrievable form became a topic of study with the increasing popularity of processing theories in the 1970's.

The vast majority of research on the behavioral characteristics of Korsakoff patients during the 1970's concerned their total inability to learn and remember any new information. This characteristic completely overshadowed, and perhaps contributed to, their other defining characteristics, such as perseveration, confabulation, and emotional passivity. Patients act as if every given moment is a new entry into the conscious world with nothing preceding it.

The major question during the 1970's concerned the nature of this acquisition disorder. Some investigators, such as Warrington and Weiskrantz (1970, 1974), felt that the problem represented a pure retrieval deficit. The brain apparently represented the material somewhere, but the patient could not find it. Others, including the author (Cermak 1979; Cermak et al. 1973), believed that the disorder was produced by an encoding deficit, an initial inability to adequately process the material so that later retrieval would be possible.

Much of Warrington and Weiskrantz's evidence for retrieval difficulties came from a series of cueing studies. In these studies, the patients were shown a short list of words, then given a yes/no recognition test in which the words from the list were interspersed with new words. Finally, they were required to tell whether a particular word had been on the list. The patients performed at chance levels on this task. On a subsequent task, patients were given a three-letter cue for each word on the list and told to try to use the cue to remind them of the words. This cue reminded the patients of the words on the list as often as it did for the controls; that is, amnesic patients recalled as many list words as normals. This finding suggested that the word had been placed into memory but could not be retrieved by amnesic patients on their own.

Other inquiries leading to the same conclusion involved the use of degraded pictures. In these studies, patients were shown partial line drawings of common objects. Gradually, more of the figure was completed until the patient could identify the item. Amnesic patients could identify the figure with as little information as normals. What was of greater import was that the patients needed less input the following day to identify the same figures, although they vehemently denied ever seeing them before. Clearly, some memory for the information had been retained, although it was not enough to produce accurate recall. Warrington and Weiskrantz concluded that the material had been encoded into memory adequately, but that the patient's retrieval of the material was impaired. Given a partial cue, the patients performed better than expected compared with the prior day's

performance. Therefore, the item must have been in memory.

Evidence for a processing, or encoding, interpretation of alcoholic Korsakoff's amnesia came from a series of experiments performed by Cermak and Butters (for a complete review see Butters and Cermak 1980). In the first of these, the patients were shown a short list of eight words. Two of the words were names of flowers, two were tools, two were body parts, and two were professions. After the list had been read, the patients were asked immediately to try to recall the words. This type of recall essentially measures memory span. On this task, patients performed normally (both groups remembered 4 to 5 words). However, if instead of allowing this type of rote recall, they were asked to recall the list by category (first the body parts, then the flowers, etc.), they were unable to recall the list. Normal controls, on the other hand, profited from these recall cues and remembered 7 to 8 items. The semantic cues helped the normals but hindered the amnesics.

We interpreted this outcome as reflecting the amnesic's inability to initially analyze and store (i.e., encode) the semantic features of the words. Because the items had not been stored in this organized fashion, they had no mechanism available at the time of retrieval to sort by category. Of course, such an interpretation based solely on this one experiment seems presumptuous. However, the evidence from other studies done by us during the early 1970's pointed in the same direction, and other investigations studied the phenomenon more directly.

For instance, in an experiment on encoding, Cermak et al. (1974) employed a paradigm pioneered by Wickens (1970). In this procedure, which has been called the "release from interference" task, patients are shown three words, all belonging to the same taxonomic category (e.g., animals). Subjects are required to remember the items over a 20-second interval. During this interval, subjects are prohibited from rehearsing the items because they are required to name colors presented at the rate of one per second. At the end of the interval, subjects attempt to recall the words.

For the most part, the alcoholic Korsakoff patients performed well on this (first) trial, remembering about 90 percent of the information. Only an occasional patient forgot any of the three words. However, the patients then received a second trial of words to recall. These words were also from the category of animals. When recall of this second set was required after 20 seconds, retrieval had degenerated to about 65 to 70 percent for all subjects. By the time a fourth trial of words had been attempted, retrieval was quite poor. The performance of normals degenerated to approximately 50 percent recall, amnesics to around 20 percent. Apparently, similar material from trial to trial interfered with the subjects' ability to recall items on any specific trial.

What happened next, however, was the critical part of the experiment. On a fifth trial, the examiner presented three words from a taxonomic category other than animals (e.g., vegetables). Retention on this trial returned to 90 percent for the

controls, but did not do so for the amnesics. Instead, their retention remained at 20 percent. The normal controls seemed able to automatically detect and use the taxonomic change to aid their storage and ultimate retrieval. Thus, for normal controls, one class of material did not interfere with the retrieval of another. The Korsakoff patients, on the other hand, were not able to automatically perform such analysis and encoding. The new material interfered with their performance at the time of recall, regardless of class inclusion. Given enough time, patients could separate the words into appropriate classes; however, within this specific paradigm, they could not perform quickly and automatically. From this outcome, we concluded that alcoholic Korsakoff patients have an encoding impairment.

Processing theory received considerable impetus in 1972 with the publication of a theory by Craik and Lockhart called "Levels of Processing." This theory held that the "depth" to which information was initially processed determined the probability that an item would later be recalled. Depth was loosely defined in terms of the cognitive analysis performed on the material and was demonstrated in several different ways. For example, if subjects were instructed to analyze the semantic characteristics of a word, they were more likely to remember the word later than if they were asked to analyze only the phonemic characteristics. This hypothesis spawned a decade of research led by processing theorists. They were eager to show that it was the nature of processing that determined the life span of the memory of a piece of information, rather than the paradigm of putting information into short-term or long-term memory. It was shown that semantic analysis produced a memory with longer life span than did phonemic analysis.

In a series of experiments based upon the Craik and Lockhart thesis, we found that alcoholic Korsakoff patients were simply unable to profit from deeper analysis of incoming information in the same way as normals. We knew already that these patients did not automatically analyze the semantic features of verbal information. What we showed is that even when they could be induced to perform such analysis, a lasting memory did not result.

Adapting a paradigm introduced by Craik and Tulving (1975), we presented a short list of words to our patients for later recall (Cermak and Reale 1978). For some of these words, the patient was asked to respond yes or no as to whether the word was written in upper case (orthographic level of analysis). In another condition, we asked the patient whether the presented word rhymed with a particular word (phonemic level). Finally, we asked if the word fit into a particular sentence (semantic level). The response speed for the patients to answer these questions was found to be normal. When we looked at the level of answering the questions correctly, they were again normal and, for that matter, performed almost perfectly.

Only when we looked at their retention for this material did we find a different pattern. The level of retention of

controls was solely a function of the depth of analysis. Phonemically analyzed words were recalled significantly better than orthographically analyzed words. Similarly, semantic processing was better than phonemic processing. On the other hand, retrieval for amnesics did not vary at all as a function of encoding depth. Retrieval remained at approximately 15 percent whether the material was analyzed by case, rhyme, or sentence. Normals' retention also began around 15 percent for case, but rose to 45 percent for rhymes, and to nearly 65 percent for sentence. Although amnesics analyzed the words on any level required, the product of this analysis did not change because of the level.

As the decade of the 1980's emerged, the pendulum had swung from dominance by systems theories to processing theories. Even Warrington and Weiskrantz (1978) concluded that the extent to which amnesics could cognitively manipulate verbal information of storage and retrieval was deficient relative to normal controls. They still emphasized the retrieval aspects of amnesics' disability; however, they now contended that a major factor in the lack of retrieval routes available to amnesics was the extent to which they could manipulate information at the time of input.

Interestingly, it was a serendipitous finding among amnesics (known to clinicians and often portrayed as a clinical curiosity) that led to the beginning of the swing toward systems theory again. This swing culminated in the introduction of two new types of memory into the literature of normal and abnormal memory, namely "explicit memory" and "implicit memory." However, before these can be defined, the concept of "priming" an amnesic's memory system must first be introduced.

PRIMING AMNESICS' MEMORY

An early instance of priming of amnesic patients' memory was reported by Gardner et al. (1973), who called it the "out of the blue" phenomenon. After these investigators presented a list of words to patients and asked for recall, they then had the patients free-associate to the name of a category of words (e.g., automobiles). They found that patients could be induced to respond to this category cue with an exemplar from the just-presented list at a rate much higher than anticipated from free-association norms. In other words, the patient would respond to the cue of automobile with the word "Buick," which happened to be in the list, as one of their first associates. Clearly other exemplars would normally be first associates; however, the patient had been "primed" in some manner by the inclusion of that particular exemplar in the list, although the term could not be recalled.

Priming became more formalized in a much later study by Graf et al. (1984) that used a procedure known as word-stem completion. The examiner presented a list of words to the patient who was instructed to remember them for later recall. Then at the time of recall, the patient was shown a three-letter cue (the first three letters of the word) and asked

to complete the word. Under one set of instructions, the patient was told to complete the word with one that had been in the list. Under a second set of instructions, the patient was told simply to respond with the first word that came to mind, and no reference to the list was made. Graf et al. found that amnesic patients performed significantly worse than the normal controls under the usual cued recall conditions. However, they performed normally (i.e., filled in the word stems with words that came from the just-presented list) when simply asked to respond with the first word that came to mind. Thus, when the instructions for the test were phrased in terms of a recall test, the patient was impaired. On the other hand, when no reference was made to the to-be-retained list, the patient's performance was normal. This pattern seemed to show that some memory for the items was present.

A similar phenomenon was reported by Cermak et al. (1985) using a completely different procedure. In their task, patients were also shown a list of words and then asked to try to remember them, this time using a recognition procedure. As expected, recognition was at chance levels, far below the level of the normal controls. The patients were then placed into an entirely different task situation. They were asked to identify words presented on a computer screen at durations that were below the patients' thresholds. Duration was then increased for each word until the patient could identify the word on the screen. Some words had been presented during the just-completed memory test, but of course they had not been recognized as such by the patients. However, these same words had an effect on performance during the perceptual identification task. The amnesic patients identified the words previously presented during the memory test more quickly than new words. Furthermore, the extent of the priming effect for the just-presented words was as great as that seen for normal controls. Thus, the phenomenon of priming became established across paradigms. Other paradigms rapidly emerged in the literature, such as those influencing "spelling bias" of homonyms and reading speed of inverted text. However, word-stem completion and perceptual identification were the two most frequently used paradigms.

It became important to provide a theoretical explanation for the phenomenon of priming in amnesia, and several theories emerged to fill the void. A popular theory presented memory as divided into two components, procedural learning and declarative learning. Championed by Cohen and Squire (1980), this theory proposed that amnesics have normal procedural memory but impaired declarative memory. Procedural memory was defined as knowing how to do a task, whereas declarative memory was defined as knowing that a certain fact had been learned. Because amnesics can learn to perform some skills (such as negotiating a finger maze or acquiring the correct pattern to solve the Tower of Hanoi task), Cohen and Squire (1980) proposed that amnesics can learn and retain procedures needed to do a task but cannot acquire declarative knowledge of this act.

They extended their theory to include performance on implicit memory tasks by pointing out that the nature of most of these tasks requires that certain perceptual operations or procedures be repeated within the same experiment. In the perceptual identification task, patients have to see and pronounce a word on a computer screen when it is first presented in the acquisition portion of the experiment. Then they try to see and pronounce the word during the identification portion of the experiment as well. The similarity in procedure, both in terms of stimulus presentation and task requirements, is the same. Thus, the patient can perform adequately using intact procedural memory. Failure to explicitly recognize the words occurs because it requires the use of declarative memory. Thus, the procedural/declarative theorists used the data from priming performance to support their distinction.

Similarly, proponents of an alternative memory system theory, the dichotomy between episodic and semantic memory, also found a way to incorporate this phenomenon into their theoretical distinction. Initially described by Tulving (1972, 1983), episodic memory was defined as the ability to retain and retrieve an item within the context in which it was originally presented. Semantic memory was defined as a context-free system in which individuals retained facts and knowledge without reference to the context in which they were learned. Theoretically, semantic memory had its own internal organizational structure in which information was represented as similar bits of information (i.e., in some manner all animals are represented within their taxonomic category). Recall from both systems required that a subject realize that they know a particular item of information is correct. Therefore, the systems were not seen as directly superimposable upon the procedural/declarative distinction. Instead, the episodic/semantic distinction was viewed as nested within the declarative system (Cermak et al. 1985; Tulving 1985).

Amnesics have relatively normal semantic retrieval, but below normal episodic retrieval (Kinsbourne and Wood 1975; Cermak 1984). Impaired explicit task performance occurred because the patient was being asked to provide an item in context ("Was the item on the just-presented word list?"). Such contextual information was not available to the amnesic because it would be in episodic memory. Their normal implicit memory performance (priming), on the other hand, was supported by their normal semantic memory, because the word's presence on the original to-be-retained list was seen as producing "activation" of that item's representation in semantic memory. This activation sensitizes the item's representation, making it temporarily more accessible the next time the stimulus is presented or partially presented. Thus, the initial presentation "primes" the accessibility of that same item on an implicit task, such as perceptual identification or word-stem completion. Thus, two different memory systems theories used the same findings as evidence for their particular theories.

ACQUISITION OF NOVEL INFORMATION IN IMPLICIT MEMORY

The theory that a stimulus item contacts semantic knowledge to produce normal implicit memory in amnesics clearly suggested that new implicit learning would not be possible for amnesics, just as new explicit learning was not possible. However, Graf and Schacter (1985) showed that new learning could occur in an implicit memory task combining word-stem completion and paired associate tasks. In this task, a novel association between two words (e.g., window - reason) clearly not in semantic memory before the task, appeared to acquire enough strength to result in what they called contextual word-completion performance by amnesics. In this task, completion of the word-stem "rea---" was significantly higher in the presence of its learned associate "window" than when presented alone or with another unrelated stimulus. This meant that activation of already acquired associative information was not necessarily essential for amnesics' implicit task performance. Apparently, the episode itself affected the patients' performance.

Attempts to replicate the Graf and Schacter result for other amnesics have fallen short, and we have not been successful in replicating it for alcoholic Korsakoff patients. This initial finding did stimulate investigators to question whether subject-initiated analysis during item presentation might potentially have some effect on subsequent implicit performance, a suggestion that provided the impetus for many recent investigations of processing in implicit memory.

For instance, Moscovitch et al. (1986) found that amnesic patients could read pairs of words faster upon a second presentation, even when the word-pairs were presented on a computer screen in a degraded fashion. They attributed this to the patients' implicit retention of the word-pair, which enabled them to read the second member of the pair faster when it could be anticipated than when it could not.

Schacter et al. (1984) found that amnesic patients acquired novel information, such as "Bob Hope's father was a fireman," but could not remember where they had learned it. In fact, they had heard it from the examiner during the experiment. However, they did not realize this and tended to attribute this knowledge to some hypothetical external source or simply assumed that it was common knowledge.

Johnson et al. (1985) reported that amnesics acquired preferences for melodies they had already heard during an experimental session. The task involved asking amnesics to judge whether they liked certain melodies. The amnesics liked the melodies just presented in a memory portion of the experiment better than they liked ones that had not. Again, this outcome occurred despite their inability to recognize which items had been previously presented.

Crovitz et al. (1979) noted that the time required by amnesics to find hidden figures in pictures was progressively reduced with experience with the same pic-

tures, even though the patients did not realize that pictures were being repeated. Thus, amnesic patients are clearly able to profit from prior experience when the material under investigation is new or novel for them. Amnesics do not necessarily profit as rapidly or completely as normals from this prior experience with new material, but some effect of prior experience on behavior seemed to be occurring. It has been suggested that amnesics do not profit as much as normals because normals accomplish more extensive processing of the novel material at the time of presentation than amnesics (Cermak et al. in press). Consequently, normals are probably more aware of their learning than the amnesics and can use this awareness as an adjunct to the purer implicit process relied upon by amnesics. This suggestion has been incorporated into yet another dichotomy of amnesics' abilities and inabilities, which, as might be expected by this time, provides yet another explanation of the explicit/implicit task performance differential seen in amnesia. This dissociation has been called the aware/unaware memory distinction.

CONTEMPORARY PROCESSING THEORIES

Mandler (1980) and Jacoby (1984) were among the first to suggest that two different forms of initial information processing might exist simultaneously for normals, and that one of these forms of processing might contribute to amnesic patients' normal performance on implicit tasks. Since the emphasis in this theory is on processing during the episode itself, Cermak et al. (1985) have proposed that this dichotomy does not superimpose upon either the semantic/episodic or the procedural/declarative; instead, it seems to be a dissociation within episodic memory.

At the time of initial stimulus presentation, normal subjects perform some processing of which they are aware and that they retain to help reconstruct the episode on an explicit memory task. It is this aware-processing that is not available to the amnesic.

The second type of processing occurs automatically and includes such aspects as perceptual analysis of the visually presented word. This type of processing does not aid reconstruction at the time of recall but probably facilitates faster processing on an implicit measure, such as perceptual identification, although the subject is never aware that this processing is occurring. This unaware level of processing may be available to amnesics and would support their normal implicit performance. Thus, amnesic patients' intact processing could exist on a purely automatic perceptual level, which may be sufficient to support implicit, but not explicit, memory.

Strategic or conceptual processing, which probably forms the basis for aware memory, may not be available to amnesics. Thus, at the time any memory task (explicit or implicit) is performed, normals have more processing available than amnesics. Therefore, they do better on implicit tasks involving new learning, such as those described above.

To explore the possibility that amnesics' implicit memory is not entirely normal on all processing dimensions,

although their performance on tasks designed to assess implicit memory is normal, tasks were developed to investigate perceptual and conceptual processing abilities during what would typically be called an implicit memory task. Consequently, our next series of tasks was based on Jacoby's studies of perceptual (perhaps automatic) and conceptual (strategic) analysis.

Our first attempt used a perceptual identification task with novel information. We had previously observed that amnesics did not profit from repetition of pseudowords (words such as "broge") in this procedure to the extent that normals do (Cermak et al. 1985). Our interpretation was that the below-normal priming for novel material occurred because the pseudowords lacked semantic representation and thus could not be sensitized during initial item presentation. However, another important difference between pseudowords and real words is that pseudowords have an unfamiliar orthography and phonology. To determine whether deficits in analyzing these features could also account for the difference between amnesics' real-word and pseudoword priming, we did two more experiments using this perceptual identification procedure (Cermak et al. 1991).

These studies used stimuli that share a phonology with real words, but are not themselves real words and thus have no existing orthographic representation. We called these stimuli pseudohomonyms (e.g., phaire). We found that amnesics could be primed for these items at the same level as for real words, comparable to normals. We hypothesized that the pseudohomonym was contacting the corresponding real word's representation in semantic memory, assuming that the pseudohomonym did not have its own independent representation in memory. Of course, it was possible that the stimulus was being "learned" in one trial parallel to those studies cited above.

Further study suggested that this latter hypothesis was not accurate, and that the contact with the real word representation did not occur automatically for the amnesic. We found this by performing an experiment in which pseudohomonyms and pseudowords were both presented within the same list of stimuli and assessed on the perceptual identification task in the same mixed fashion. When this was done, the pseudohomonym priming effect disappeared entirely, and neither pseudohomonyms nor pseudowords showed any priming. Apparently, the relationship between real words and pseudohomonyms was not readily apparent to the patient.

The mixed-list design showed both that this contact did not occur automatically upon each presentation of a pseudohomonym, and that it did not represent an ability to learn this type of material at first presentation. Instead, it appeared that this type of processing depended upon the patients' realization that the pseudohomonyms corresponded phonemically to real words. This realization occurred when all the stimuli shared this characteristic, but not when stimulus types were mixed within a list. Thus, when conceptual processing is required,

amnesics may not spontaneously accomplish such controlled processing, failing to exhibit normal implicit performance under these conditions.

This proposal must strike the reader as highly similar to that suggested for explicit memory. Previously, we have seen that although Korsakoff patients can analyze semantically, on their own they do not perform much analysis. Consequently, they cannot reconstruct the verbal information at retrieval. What the current experiment suggests is that under conditions where amnesics do not perform spontaneous conceptual encoding of an item's features at input, their implicit memory stands to suffer as well. Amnesics may be capable of perceptual processing because it is almost automatic. However, they are not capable of conceptual processing because controlled processing beyond the automatic level is required. Those implicit tasks that depend upon perceptual processing for successful performance may be performed normally by amnesics. Those that depend upon conceptual processing may not be performed normally when the patients are on their own to do the necessary processing. It became important to separate the effects of automatic processing and controlled processing on implicit task performance to assess the contribution of each to the performance of amnesics and normal subjects.

To accomplish this goal, we have recently created a situation in which the effects of automatic processing and conscious processing directly oppose one another in the performance of a task using a paradigm developed by Jacoby and Kelley (1991). In this paradigm, patients performed a word-stem completion task after being specifically instructed *not* to use the words that had just been presented on the study list. Under these exclusionary instructions, we (Cermak et al. 1992) found that amnesic patients completed more word-stems with list items than normal controls. In fact, when we compared performance on this task with that on a standard word-stem completion task, the exclusion instruction had very little effect on the performance of amnesics, whereas it sharply decreased the number of study words used by controls. It appeared that the Korsakoff patients were unable to attribute the familiarity of the first word that occurred to them as resulting from the list that had just been presented. This may have been due to the amnesic patients' reliance on their initial automatic analysis of the physical features of the words without any further conceptual analysis. When the word-stem appeared, the automatic analysis proceeded easily, resulting in word generation that should have been inhibited by the specific instructions provided. Controls used their initial conceptual analysis of the word to provide a check on the fact that the word had just been previously analyzed and therefore could be excluded from use on the present task.

In a supporting experiment (Cermak et al. in press), which was also designed to oppose the fluency generated by automatic processing with recollection based on controlled processing, using a list of names randomly selected from a phone

book, amnesic patients were asked to pronounce each name as it was presented. Then, using another list of names, patients were asked to judge the fame of each name that was presented to them. Some of the names in this latter task were indeed famous, although not to the point of being immediately identifiable (e.g., Simon Bolivar, Henry Thoreau). All of the nonfamous names were taken from the phone book, and half had been presented during the pronunciation task that had just been performed and half were new to this experiment. The patients were assured before this fame judgment task that the names that they had just pronounced were randomly drawn from a phone book and were definitely not famous; therefore, should one occur during the fame judgment task, and some definitely would, the patient could confidently respond "not famous." As in the word-stem exclusion task, we found that the amnesic patients were much more likely to endorse an old, rather than a new, nonfamous name as famous, whereas controls did not. These results suggest the hypothesis that amnesics are unable to use conscious processes to oppose the automatic effects of memory.

We next asked whether amnesics would also fail to use controlled processing to enhance the effects of automatically generated fluency by testing for the effects of conscious recollection and fluency when they were not opposed. Patients were told that the names just presented on a pronunciation task were famous (albeit obscure) and ought to be responded to positively in the fame judgment task (we called this an "inclusion" condition). In contrast to the exclusion condition, we found that the normal controls endorsed significantly more old nonfamous names as famous than new nonfamous names and significantly more than the Korsakoff patients. In fact, amnesic patients endorsed no more old names as famous than they had on the exclusion condition.

All these data suggest that Korsakoff patients' performance was mediated almost exclusively by the effects of the fluency generated by repeated automatic processing of the perceptual features of the stimulus item. Controls, on the other hand, used conscious recollection either to enhance or to counteract the effects of this processing fluency. The controls could recognize the source of their fluent processing as coming from a prior presentation, largely because they had analyzed that prior occurrence on a level beyond automatic perceptual processing.

PROCESSING THEORY AS ORTHOGONAL TO SYSTEMS THEORY

At this point, at least four dichotomies of memory have been defined, using the performance of amnesics on implicit priming tasks to substantiate the existence of two distinct memory systems. These include procedural/declarative, episodic/semantic, aware/unaware, and perceptual/conceptual. The underlying problem has been that there is no absolute way to differentiate among these dichotomies, since they all predict amnesics' performance in the same direction. Recently, however, Roediger (1990) has suggested a solution

to this dilemma. He has proposed that processing abilities might cut across memory systems and make different predictions within memory systems depending upon which type of processing is used. The processing dimensions he considers to be orthogonal to the explicit/implicit dimension are called data driven and conceptually driven.

Data-driven processes are those that occur automatically when the stimuli are presented and include such processes as perceptual, phonemic, and auditory. Conceptually driven processes are initiated by the subject and include such processing as semantic, reflective, and organizational. These processing abilities are perceived as orthogonal to explicit/implicit memory systems because they exist within both components. In other words, there could be data-driven implicit or explicit performance. Also, there could potentially be conceptually driven implicit or explicit performance. The mistake that investigators have been making, according to Roediger, is that they have been focusing only on the data-driven implicit and the conceptually driven explicit categories. He suggests that tasks should be developed to examine the remaining categories, i.e., data-driven explicit performance and conceptually driven implicit performance. Such a development must reveal that amnesic patients are impaired, not along the explicit/implicit dimensions, but along the data-driven/conceptually driven dimension.

The difficult aspect of this theory is to devise creative paradigms that examine these categories. Roediger believes that this objective can be obtained. He suggests two alternatives: (1) a sound-alike cue given during recall as a data-driven explicit task, and (2) a trivia-like question delivered during a priming portion of an experiment as a conceptually driven implicit task.

This type of analysis with amnesic patients has not yet been performed. The outcome presented above, however, showing the difference between the effects of fluency and reconstruction on amnesics' task performance, seems to be based on a differential ability to do perceptual versus conceptual processing. The studies reviewed in this chapter suggest that perceptual processes are usually automatic and therefore are preserved in amnesia; whereas the conceptual processes are more strategic and are impaired in amnesics. The terminology is not identical, but the meaning is the same as that proposed by Roediger. It appears that a processing ability dimension might cut across memory systems, with amnesics capable of one type of processing (automatic) but not the other (strategic).

In the future, systems theorists and processing theorists will compare tests that directly assess the performance of amnesics on those categories proposed by Roediger. This empirical process will enable us to determine whether the deficits observed in amnesic alcoholics are due to differences between systems of memory or to their ability (or inability) to perform specific types of processing, whatever the memory tasks. If, as predicted here, the latter appears more likely, then the pendulum swing between

systems versus processing will return to the side of processing functions as the focus for the study of amnesic memory disorders.

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8

IMPAIRED NEUROPSYCHOLOGICAL COGNITIVE FUNCTIONING IN SOBER ALCOHOLICS

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INTRODUCTION

Ample biomedical evidence, detailed in other chapters in this book, indicates that detoxified, sober alcoholics have altered brain structures and functions. There is equally compelling evidence that sober alcoholics, when compared with peer nonalcoholics, have decrements on measures of intellectual functions of a cognitive, perceptual, and perceptual–motor nature. These decrements have been studied systematically over the last three decades by neuropsychologists.

Neuropsychology, as a discipline, is concerned with the relations between brain and behavior, especially the relation of brain structures and functions to measures of various aspects of mental functioning. The basic principles of human neuropsychology have been derived largely from the performance of patients with known brain lesions or disorders on tests of mental functioning. Characteristic patterns of cognitive deficits have been established, for example, deficits associated with frontal, left hemisphere, right hemisphere, bilateral or generalized, and subcortical damage or dysfunction. Tests measuring such cognitive deficits are termed neuropsychological tests. They are used to characterize the deficits in other disorders in terms of possible brain areas involved and the extent and severity of the dysfunction.

The neuropsychological changes in sober alcoholics range from no discernible residual impairment, through mild to moderate impairment in one or more functions, to clinically diagnosable brain syndromes, such as the amnestic syndrome (Korsakoff's disorder) and alcoholic dementia. Approximately 10 percent of sober alcoholics fall into the latter two diagnostic categories (Horvath 1975). The remaining 90 percent of the patients do not meet the criteria for organic brain syndrome (see next section for details), yet most of them show some cognitive changes as noted earlier. For years, some clinicians have described these alcoholics as falling into an intermediate stage of alcoholic brain disease (Bennett et

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al. 1960). More recently, Grant et al. (1987) proposed a new diagnostic category for such patients: intermediate duration (subacute) organic mental disorder of alcoholism. While I believe that their proposed diagnosis is a more apt characterization, I will, for purposes of brevity, refer to this proposed diagnostic category as intermediate stage alcoholics.

In the remainder of this chapter, I will first briefly consider the pattern of cognitive deficits in the diagnosable organic mental disorders of alcoholism. Next, in the major focus of this chapter, I will describe the status of research on the neuropsychological deficits and recovery from them in intermediate stage alcoholics. A final summary and conclusions section follows.

NEUROPSYCHOLOGICAL CHANGES IN PATIENTS WITH ALCOHOLIC ORGANIC MENTAL DISORDERS

In the DSM-III-R (American Psychiatric Association 1987), amnestic disorder and alcoholic dementia are separable diagnoses. In practice, these two syndromes are very difficult to separate on neuropathological grounds. Adams and Victor (1989), based on their extensive experience with these syndromes, report that most cases coming to autopsy with the diagnosis of alcoholic dementia or alcoholic deteriorated state have the same lesions that characterize the Wernicke-Korsakoff syndrome. There are very few studies of alcoholic dementia, but many studies of the amnestic syndrome characteristic of Korsakoff's disorder.

The striking changes in retrograde and anterograde memory in Korsakoff patients and many other aspects of the disorder are examined in detail in other chapters by Cermak (chapter 7) and Martin and Nimmerrichter (chapter 23). Therefore, in the present chapter, discussion of this disorder will be limited to several aspects of how the clinical neuropsychological approach has contributed to our understanding of it.

The first point is that neuropsychologists have identified and systematically measured not only the memory impairments but many other cognitive deficits present in Korsakoff patients. As long ago as 1965, Talland devoted a monograph to describing these changes. He gave Korsakoff patients and controls a broad battery of tests measuring intelligence, reasoning, memory, and perceptual and problem-solving functions. Although the Korsakoff patients' IQs were in the normal range and did not differ from the controls, the patients had the expected severe deficits in memory. They also displayed moderate to severe deficits on the other cognitive tests, especially those measuring abstracting and problem-solving functions. One issue in Korsakoff neuropsychological research is whether these other deficits are secondary to a primary impairment in memory.

Janowsky et al. (1989) investigated this question using memory tests and the Wisconsin Card Sorting Test (WCST), a problem-solving sorting test in which the patient must shift his or her basis of sorting from one attribute to another. The tests were given to four groups of patients: patients with frontal lobe resections, Korsakoff patients, non-Korsakoff amnesic

patients, and controls. The Korsakoff and non-Korsakoff amnesic patients were impaired on the memory tests, but only the Korsakoff patients and frontal lobe patients were impaired on the WCST. The fact that the non-Korsakoff amnesics had the expected memory deficits similar to the Korsakoff patients but did not have impairment on the problem-solving task suggests that the Korsakoff cognitive difficulties are more extensive than just memory problems. Joyce and Robbins (1991) made a similar conclusion after studying planning and spatial working memory in Korsakoff and non-Korsakoff alcoholics.

There is a second point to make about the contribution of neuropsychology to the Korsakoff disorder. The research cited above and other neuropsychological studies conducted by Butters' laboratory (Butters and Gramholm 1987) have strongly suggested that Korsakoff patients not only have memory deficits associated with the known lesions of the mammillary body and dorsal-medial nucleus of the thalamus, but also have a pattern of cognitive impairment suggesting dysfunction in the frontal-basal areas of the brain. These kinds of findings both broaden our understanding of, and stimulate further research in, Korsakoff's disorder.

NEUROPSYCHOLOGICAL DEFICITS IN INTERMEDIATE STAGE ALCOHOLICS

Studies Comparing Alcoholics and Brain-Damaged Patients

There are scores of studies of neuropsychological deficits in intermediate stage alcoholics, but four studies in particular have helped to establish the tie of cognitive deficits to brain dysfunction. Each study has compared the neuropsychological performance of alcoholics to patients with known brain damage due to other causes, such as strokes, tumors, and head trauma. These studies, each with 30 subjects or more in each group, used the Halstead-Reitan neuropsychological battery (HRB), a highly reliable and repeatedly validated battery identifying deficits associated with brain dysfunction.

Fitzhugh et al. (1965), in the pioneering study in this area, reported that their alcoholic subjects' HRB performance was significantly poorer than that of peer controls and approached the level of performance of the group of heterogeneous brain-damaged patients. Jones and Parsons (1971) used the Halstead Category Test, an abstracting test that is one of the most sensitive HRB tests of brain dysfunction, to compare triads of age- and education-matched alcoholic, brain-damaged, and control patients. Alcoholic and brain-damaged patients had similar levels of impaired performance compared to controls. Miller and Orr (1980), using the whole HRB, found that both alcoholics and a heterogeneous group of brain-damaged patients performed poorer than controls on 18 HRB-derived measures. However, the alcoholic subjects and brain-damaged patients did not differ on 17 of the 18 measures. Alcoholics' performance ranged from mild to severely impaired, but averaged in the moderate range. Finally, Goldstein and Shelly (1982) compared alcoholics

with diffuse brain-damaged patients on the HRB plus other tests and found very similar levels of performance. On an overall HRB index of impairment, both groups' average performance was in the moderately to severely impaired range.

These studies provide convincing evidence that sober intermediate stage alcoholics, usually tested 2 to 4 weeks after the acute withdrawal symptoms have subsided, manifest neuropsychological deficits equivalent to those seen in mildly to moderately, and sometimes severely, brain-damaged patients. Given these findings, there are several important questions to be answered: Which cognitive abilities are most impaired, what are the determinants of individual differences in the cognitive deficits, and what is the course of recovery of cognitive functions?

Cognitive Abilities Impaired in Intermediate Stage Alcoholics

Numerous studies from laboratories throughout the Western World have reported cognitive deficits in alcoholics compared to peer nonalcoholic controls. Reviews of these studies (e.g., Chelune and Parker 1981; Eckardt 1981; Miller and Saucedo 1983; Parsons 1987; Parsons 1989) have reported deficits, such as impaired episodic memory, difficulties in learning new material, poorer abstracting and problem-solving abilities, slower perceptual-motor performance, impaired visuospatial abilities, and slowed speed of information processing.

Studies from our laboratory over the last two decades have contributed to these conclusions. In our most extensive study to date (Parsons et al. 1990*b*) we gave a battery of neuropsychological tests to male and female alcoholics and community control subjects. The groups averaged in their midthirties and had 12 to 13 years of education. Alcoholics, recruited from community treatment programs, had been detoxified for a minimum of 3 weeks. All met DSM-III-R criteria for alcoholism and were carefully screened for any other medical or psychiatric disorders that might affect brain functioning. Control subjects were recruited from the community by newspaper ads.

Given the similarity in factor structure across the groups, the neuropsychological performances for the subjects were collapsed across groups using a principal components, varimax rotation factor analysis. Four factors were found. The tests comprising each factor are found in table 1. Factors 1 and 2 reflect the well-known division of verbal and visuospatial abilities. Factor 3 clearly is composed of perceptual-motor tests, and factor 4 uniquely measures memory for paragraphs.

Comparisons of the alcoholics and peer controls are presented in table 2. Age and education-corrected *T* scores (mean of 50 and standard deviation of 10) for each test were averaged for the tests in each factor. As can be seen, alcoholics performed significantly poorer than controls on all factors. Note that despite using age- and education-corrected scores, the alcoholics performed much poorer than controls on the verbal factor. This finding suggests that psychological processes (verbal-linguistic), usually asso-

ciated with integrity of the left hemisphere functioning, are as impaired as those psychological processes (visuospatial) usually associated with integrity of the right hemisphere. Such a conclusion supports the "diffuse" model of the effects of chronic alcohol abuse upon the brain (Parsons 1987).

In a more recent series of studies, we investigated the "efficiency" of alcoholics, that is, accuracy or correctness of performance per unit of time. Theorists of intelligence have long recognized the importance of time or speed of performance as a dimension of intelligent behavior (Cattell 1963; Hunt 1978; Jensen 1980; Sternberg 1984). In life situations, such as work, time taken to perform a task is often a critical variable. There is little doubt that the person who can satisfactorily accomplish a task in less time than other individuals is a preferred employee.

Most of the tests of cognitive functioning comprising our battery are not

TABLE 1

Tests comprising four factors

Factor 1 Verbal
- Shipley Vocabulary (Shipley 1940)
- Word Finding Test (Reitan 1972)
- WAIS-R Information (Wechsler 1981)
- WAIS-R Comprehension (Wechsler 1981)
- Conceptual Level Analogies (Willner 1970)
- Shipley Abstracting (Shipley 1940)
- Face-Name Paired Associates (Schaeffer and Parsons 1986)

Factor 2 Visuospatial
- Wechsler Memory Scale Figural Immediate (Russell 1975)
- Wechsler Memory Scale Figural Delayed (Russell 1975)
- WAIS-R Block Design (Wechsler 1981)
- Booklet Category Test (DeFillipis and McCampbell 1979)
- Symbol-Digit Paired Associates (Ryan and Butters 1980)

Factor 3 Perceptual-motor
- Pegboard—Nondominant (Rennick et al. 1972)
- Pegboard—Dominant (Rennick et al. 1972)
- WAIS-R Digit Symbol (Wechsler 1981)
- Trails A (Rennick et al. 1972)
- Trails B (Rennick et al. 1972)

Factor 4 Semantic memory (logical memory)
- Wechsler Semantic Immediate (Russell 1975)
- Wechsler Semantic Delayed (Russell 1975)

Notes: All tests loaded at least 0.40 on their respective factors; the median factor loading was 0.72 across the four factors.

TABLE 2

Group means for the four factors and ANOVA tests for differences

Groups	Factors			
	Verbal	Visuospatial	Perceptual-motor	Semantic memory
Alcoholic (n = 142)	48.51	48.83	48.95	48.24
Controls (n = 97)	52.18	51.71	51.54	52.55
F test =	14.69	8.30	7.67	10.88
p =	0.0002	0.004	0.006	0.001

timed, but fortunately we had recorded the time taken to complete the total battery of tests in the study presented above. We were then able to compare the four groups (male and female alcoholics and male and female peer controls) on accuracy or correctness of performance, time score, and efficiency index (Glenn and Parsons 1990). Accuracy scores were computed by converting all scores on the tests to T scores and obtaining the average over all of the tests for each individual. The time score was the total time taken to perform the battery less any breaks, rests, or unusual delays. The efficiency score for each individual was obtained by dividing his or her mean accuracy score by the total time.

The differences between the alcoholics and controls on these three measures were all highly significant (p's < 0.0001). Several other interesting findings were noted: Males and females did not differ on the efficiency index, and the efficiency index correlated with age in the controls ($r = -0.447$, $p < 0.001$) and alcoholics ($r = -0.203$, $p < 0.05$). The difference in correlations between controls and alcoholics was significant ($z = 2.50$, $p < 0.01$). The controls show the expected drop in efficiency with increasing age; the alcoholics show much less of a relation, although their mean efficiency scores were well below those of the controls. One possible explanation is that the individual differences in alcohol-related effects reduced the correlation. Incidentally, these findings certainly do not support the lingering notion in the literature (Kramer et al. 1989) that alcoholism results in premature aging. If the latter were the case, we would have expected higher negative correlations between efficiency scores and age in the alcoholics than in the controls.

Three other findings are of note. First, the symptoms of depression as measured by the Beck Depression Index were weakly, but significantly, correlated with the efficiency index in the alcoholics ($r = -0.285$,

$p < 0.01$), but the two variables were uncorrelated in the controls. This correlation fits with the notion that depressed individuals are less cognitively efficient (Sweet et al. 1992). However, it should be noted that the mean scores for the alcoholics on the Beck Depression Index were 8.65 (controls had a mean of 3.30), slightly below the clinically significant range of 10 to 18 for mild to moderate depression (Beck and Beamesderfer 1974). The second finding was that the female alcoholics, despite a significantly shorter duration of alcoholism ($t = 3.02$, $p < 0.003$) than for the men, were as impaired as the men. This may represent a greater vulnerability to the toxic effects of alcohol on the brain in women than in men, as some investigators have suggested (Bergman 1987). The third point is that both time and efficiency scores were correlated with frequency of drinking over the last 6 months ($r = 0.246$, $r = -0.271$, p's < 0.05), respectively. In the male alcoholics, the more frequent the drinking, the longer the time taken to complete the battery and the lower the efficiency score. In the females, however, these correlations were not significant.

The measures used in these data were based on the overall average accuracy scores and the overall time. Although we tried accounting for any unusual delays, the overall time measure in the alcoholics could have been influenced by other factors, such as alcoholics talking more during and between tests. Cumulatively over the testing period, such unobtrusive delays may have unduly lengthened the alcoholics' time scores. In our next study (Glenn and Parsons 1992), using new samples of men and women alcoholics and controls, we timed each test separately from the start of instructions for the test until it was completed. Efficiency scores were calculated as previously described and means were calculated for each of the rationally grouped, four-test clusters.

As presented in table 3, the alcoholics had significantly lower efficiency scores on each cluster. Males had significantly higher efficiency scores than females, but there were no sex by group interactions. Again, separate analyses of accuracy and speed scores were significantly different between the two groups. Alcoholics took longer to perform the individual tests, regardless of type of task, and performed them less accurately.

We concluded from these experiments that alcoholics not only have poorer performance in terms of accuracy on cognitive tests, but also take significantly longer to achieve that lower level of performance. They are demonstrably less efficient. Apparently, they use a speed–accuracy tradeoff strategy of sacrificing speed for accuracy, although this sacrifice does not raise their level of performance accuracy to the level of the controls. Finally, the alcoholics' poor performance is clearly not due to the impulsivity so frequently attributed to them; indeed, in this context, the opposite is the case.

The fact that groups of sober alcoholics consistently perform poorer than peer controls on tests measuring various neuropsychological functions should not be interpreted to mean that all alcoholics are impaired. Typically, 50 to 70 percent of such alcoholics will perform poorer on

any one neuropsychological test than their peer nonalcoholic controls. Using an overall performance score derived from several tests, as many as 85 percent may perform poorer (Parsons 1986). These findings raise the question as to individual differences in alcoholics' impaired performance and the identification of the determinants of those differences.

POSSIBLE DETERMINANTS OF INDIVIDUAL DIFFERENCES IN ALCOHOLICS' DEFICITS

Alcohol Intake Variables

The most likely contributors to the individual differences in alcoholics' impaired performance are alcohol intake variables, such as duration of alcoholism, typical quantity and frequency, maximum quantity and frequency, combinations of quantity and frequency as in the Quantity-Frequency Index (QFI) (Cahalan et al. 1969), and total amount of alcohol consumed over varying periods of time. Presumably, the toxic effects of prolonged excessive alcohol intake lead to a brain dysfunctional state. This state persists after detoxification and after the acute symptoms of withdrawal have subsided. Thus, alcohol is considered causally related to the alcoholics' neuropsychological impairment. This "causal" hypothesis seems very reasonable whether viewed from a clinical or experimental point of view.

In an extensive review paper several years ago (Parsons and Stevens 1986), we assessed the status of the causal hypothesis by considering both animal and human research. Although in animals (rats and mice), relatively high levels of alcohol and a few tasks have been used, we concluded that these studies of detoxified animals have given clear evidence for a causal relationship between duration of alcohol intake and learning and memory tasks. However, studies of humans have given inconsistent and inconclusive results.

Two types of human studies have been undertaken, including studies of neuropsychological performance of long-term alcoholics (10 or more years of alcoholism) versus short-term alcoholics (less than 10 years of alcoholism). Initial studies showed promising differences between the groups, with long-term alcoholics performing poorer than short-term alcoholics. Subsequent studies could not replicate these findings (Parsons and Stevens 1986).

Correlational studies have had a similar fate. However, there are several aspects of the results that are of interest. First, when a significant correlation between alcoholics' neuropsychological performance and an alcohol intake variable is found, it is always in the direction that the magnitude of the drinking variable is negatively related to the quality of the performance. Second, several investigators, in trying to account for varying results, have hypothesized a "threshold" for alcohol's toxic effect on the brain. After the threshold is reached, the relationship between alcohol intake variables and neuropsychological performance emerges (Bergman 1987; Eckardt et al. 1978). The problem is, of course, that such a threshold would undoubtedly be the unique product of a number of genetic and environmental fac-

TABLE 3

Group means (standard deviations) for efficiency scores across neuropsychological clusters

	Test cluster			
	Verbal	**Abstracting and problem solving**	**Learning and memory**	**Perceptual-motor**
Female Alcoholics (n = 48)	0.96 (0.17)	0.97 (0.15)	0.96 (0.18)	0.03 (0.16)
Female Controls (n = 36)	1.06 (0.20)	1.13 (0.20)	1.14 (0.21)	1.13 (0.18)
Male Alcoholics (n = 42)	1.01 (0.21)	0.98 (0.18)	1.04 (0.45)	0.96 (0.20)
Male Controls (n = 29)	1.15 (0.16)	1.11 (0.24)	1.16 (0.41)	1.05 (0.22)

Notes: The higher the efficiency score (accuracy divided by time), the better the performance; all comparisons between alcoholics and their peer controls were significant by ANOVA F tests. ($p < 0.01$ or < 0.001).

tors, making prediction of deficit in any individual before testing impossible.

In the several years since our review, we have not seen any studies that have consistently replicated any one of the previously reported relationships between history of alcohol intake and performance in sober alcoholics. An example from our own work represents the findings in the field. Schaeffer and Parsons (1986) correlated history of alcohol intake variables with a neuropsychological impairment index, based on several neuropsychological tests, in sober middle-age male alcoholics. The only significant finding was that a product of the maximum quantity consumed multiplied by the frequency of maximum intake was significantly correlated with performance such that the higher the maximal alcohol intake, the poorer the performance. In our next study of a large group of male alcoholics (Sinha et al. 1989), we found no significant relations between the same drinking variables used in the previous study and neuropsychological impairment index. In our recent study of

efficiency (Glenn and Parsons 1990), out of the same drinking variables used in the two previous studies, we found only typical frequency of alcohol intake to predict efficiency scores and then only in male alcoholics.

The inconsistency in findings suggests that attempts to identify a critical alcohol intake variable or combination of such variables as predictors of neuropsychological performance in alcoholics are likely to be unrewarding. From the studies in this area, there appear to be three reasonable conclusions. First, in any one study, if a number of drinking history variables are used, it is likely that at least one drinking intake variable will be found to significantly correlate with cognitive performance. Second, it is likely that any attempt to replicate the finding in a new sample will be unsuccessful. However, the new study may well find a different alcohol intake variable to be correlated with performance. Third, any relations found will be in the direction expected, that is, the greater the alcohol intake, the poorer the performance.

Clearly, we cannot identify any history of drinking variable that will consistently be associated with neuropsychological impairment. Consequently, we must look elsewhere. Another set of variables that may be contributing to the individual differences in alcoholics' cognitive deficits is the genetic-familial-developmental background of alcoholics.

Genetic-Familial-Developmental Factors and Neuropsychological Deficits in Alcoholics

The possibility that some cognitive deficits found in alcoholics are present premorbidly has been the focus of much research in the last several decades. Studies of nonalcoholic children, adolescent, and adult progeny of alcoholic families (FH+), compared with peers from nonalcoholic families (FH–), have provided relevant evidence. Such studies have shown that FH+ individuals have poorer visuospatial learning (Garland et al. 1993; Schandler et al. 1988); reduced visuospatial performance (Whipple et al. 1988); lower educational achievement (Sher et al. 1991); greater ataxia and poorer visual scanning and attention (Tarter et al. 1989); less accurate visual-spatial figure copying and temporal ordering and slowed learning of difficult verbal paired-associates (Peterson et al. 1992); and poorer neuropsychological test performance (Schaeffer et al. 1984). In contrast, other investigators have not found nonalcoholic FH+ individuals to differ from peer FH– individuals (Alterman and Hall 1989; Bates and Pandina 1992; Gillen and Hesselbrock 1992). Reasons for these discrepancies in findings remain obscure but are undoubtedly due, in part, to differences in the characteristics of the samples.

Although the preponderance of the above studies suggests that the FH+ variable may contribute to the alcoholics' neuropsychological impairment, the evidence from experiments directly addressing this possibility has been disappointing (Alterman et al. 1987; Reed et al. 1987). Again, an illustrative example from our own work is instructive. In our first study (Schaeffer et al. 1984), we found that both alcoholic and nonalcoholic FH+ subjects performed our neuropsychological test

battery poorer than did FH– subjects. A followup study with a similar battery failed to confirm the FH+ versus FH– differences in alcoholics and controls. However, differences did emerge when we compared FH+ individuals with alcoholic fathers (regardless of other affected family members) versus FH–; the latter performed significantly better than the former (Parsons 1989). In our third study (Tivis et al., in press), again using a similar neuropsychological battery and sampling from similar populations as in the first two studies, we found no significant effects of FH+ versus FH– or the "FH+ father alcoholic" variable on the deficits in performance of alcoholics compared to controls.

Two other developmental behavior areas have been investigated as possible determinants of premorbid cognitive deficits likely to be present in alcoholics: childhood behavior disorder (CBD) and antisocial personality (ASP). Alcoholics consistently report a higher incidence of CBDs, including attention deficit disorders, hyperactivity, and conduct disorders (DeObaldia et al. 1983; Glenn and Parsons 1989; Hesselbrock et al. 1985*a*,*b*). There is also a high incidence of alcoholics who are diagnosed as antisocial personalities (Hesselbrock 1986; Hesselbrock et al. 1985*b*).

The Childhood Symptom Checklist (Tarter et al. 1977) is a self-report instrument that covers childhood behaviors and symptoms before the age of 13, such as hyperactivity, impulsivity, distractibility, aggressive behavior, lying, stealing, and other indications of CBDs. DeObaldia et al. (1983) found that alcoholics who reported a greater number of CBD symptoms on the checklist were more neuropsychologically impaired than those alcoholics who reported fewer symptoms. These findings were supported in subsequent studies (DeObaldia and Parsons 1984; Workman-Daniels and Hesselbrock 1987; Glenn et al. 1992). In all four studies, however, the relations were modest. Finally, using young adults who had a mean age of 23.7 years (compared with ages in the late thirties and early forties of the previous studies), Hesselbrock et al. (1985*b*) found no relationship between attention deficit disorder and conduct disorder subscales from the Childhood Symptom Checklist and neuropsychological performance. Again, the inconsistency in findings points to sampling problems.

The relation between antisocial personality and performance on cognitive tests has been examined in a number of studies with equivocal results (Gillen and Hesselbrock 1992; Gorenstein 1982; Gorenstein 1987; Hare 1984; Hoffman et al. 1987). There are four pertinent studies that have examined the role of ASP in alcoholics' neuropsychological impairment. Hesselbrock et al. (1985*a*) found that ASP male alcoholics performed better than non-ASP peer alcoholics on a battery of cognitive tests. However, Malloy et al. (1989), using a similar battery of tests, found that 81 percent of male alcoholics diagnosed with ASP were impaired, compared to 52 percent of non-ASP male alcoholics. Gorenstein (1987) compared alcoholics who did not have a diagnosis of psychopath with a nonalcoholic group of psychopaths and a nonalcoholic, nonpsy-

chopathic peer control group. His findings suggested that cognitive impairment in the alcoholics was related to specific deviant behaviors, such as arrests for drunken behavior or driving under the influence, as opposed to the presence of a diagnosable ASP.

In a recent experiment (Glenn et al. 1992) we have examined the relation between CBD, antisocial behaviors (ASB), affective disturbance, and neuropsychological functioning. Male and female alcoholics, sober an average of 4 weeks, and male and female nonalcoholic controls were given an extensive neuropsychological battery similar to that in table 1. None of these alcoholics or controls was diagnosed as having a current major depressive disorder, anxiety disorder, or ASP. Thus, our scales of CBD, ASB, and affective disturbances were measuring behaviors of a subclinical nature. CBDs were measured by the Childhood Symptom Checklist. ASB comprised the antisocial personality behavioral symptoms from both the child and adult sections of the Schedule for Affective Disorders and Schizophrenia (National Institute of Mental Health 1981) and the Socialization Scale from the California Personality Inventory (Gough 1969). The affective disturbances were measured by the Beck Depression Index (Beck et al. 1961) and the State Anxiety Inventory (Spielberger et al. 1970). A single score was developed for each of the three variables, CBD, ASB, and affective disturbance.

A promax factor analysis of the battery revealed five factors that represented the following cognitive functions: verbal, visuospatial, verbal memory, perceptual-motor speed, and set-shifting flexibility. A multivariate analysis indicated that alcoholics performed much poorer than did controls ($p < 0.0005$) and that there were no group by sex interactions. Therefore, males and females were combined for further analysis. Next, stepwise multiple regression analyses were conducted for each group (control and alcoholic), with the ASB, CBD, and affective variables serving as predictor variables for each of the five neuropsychological factor scores.

The results are presented in table 4. In all entries, the *F*'s reflect negative correlations between the predictor variables and the neuropsychological scores: the higher the behavioral score, the lower the performance score. For controls, the CBD factor was a significant predictor of the verbal, visuospatial, and set-shifting fac-

TABLE 4

Stepwise regression equations with significant predictors (F's) of neuropsychological performance

	Neuropsychological Factor				
Group	**Verbal**	**VS**	**VM**	**PMS**	**SSF**
Controls	CBD 6.08[1]	CBD 15.72[2]	—	—	CBD 11.25[2]
Alcoholics	CBD 3.37[3]	CBD 10.26[4]	—	—	AFF 8.09[4]

Notes: All F's presented reflect a negative correlation between CBD and affective scores and the neuropsychological factor scores. Verbal, verbal skills; VS, visuospatial performance; VM, verbal memory; PMS, perceptual motor speed; SSF, set-shifting flexibility.

[1]p< 0.05; [2]p < 0.001; [3]p< 0.10; [4]p < 0.01 .

tors. For the alcoholics, CBD was a significant predictor of the visuospatial factor and approached significance for the verbal factor. The affective factor predicted set-shifting flexibility. The ASB factor failed to be a significant predictor of neuropsychological performance for either alcoholics or controls.

Given that the CBD seemed to account for most of the variance in the controls and alcoholics, we asked whether the two groups would differ in performance levels if CBD were used as a covariate in the multivariate analyses. This analysis produced essentially the same results that were obtained in the original multivariate analyses. Alcoholics performed significantly poorer than did controls. Thus, although CBD predicted verbal and visuospatial performance in both groups, it could not account for the differences between alcoholics and controls.

In summary, we have assessed the possible contributions of premorbid variables, such as family history of alcoholism, childhood behavior disorders, and antisocial personality, to the individual differences in neuropsychological deficits in alcoholics. No consistent evidence provides support that these variables account for the neuropsychological impairment found in the alcoholics. In any one study, depending on the sample of alcoholics, one or more of these three variables may significantly correlate with neuropsychological performance. However, the amount of shared variance can only partially account for the magnitude of the differences between alcoholics and controls. Therefore, the most viable hypothesis explaining neuropsychological impairment in alcoholics is that toxic effects of alcohol adversely affect brain function.

RECOVERY OF NEUROPSYCHOLOGICAL FUNCTIONS

What is the course of recovery of neuropsychological functioning in posttreatment intermediate stage alcoholics? The reason this question is considered in a volume entitled *Alcohol-Induced Brain Damage* lies in the implications of the answer. If alcoholics do not recover to age-equivalent norms of nonalcoholic functioning, the brain dysfunction underlying the impairment is relatively permanent, similar to those alcoholics who have diagnosable organic mental disorders. If recovery does occur over time, it implies, first, that the possibility of premorbid cognitive deficits contributing in any major way to an alcoholic's current impairment is unlikely. The second implication is that either the brain dysfunctional state has improved, that the recovering alcoholic has had an adaptive reorganization of remaining intact brain systems, or that both processes are involved. Regardless of which of these three latter possibilities is correct, the important question remains, do alcoholics' deficits remit over time?

There are two types of neuropsychological studies that have typically been used in studies of recovery. The first is a cross-sectional or independent groups design, and the second is the longitudinal design or test-retest of the same group. Two of the largest cross-sectional group studies represent the findings. Brandt et

al. (1983) compared groups of male alcoholics abstinent for 1 to 2 months, 1 to 3 years, and 5 or more years. Significantly improved performance was found for the 5 or more years abstinent group, compared to the 1- to 2-month abstinent group, on short-term memory, delayed visual-spatial memory, and psychomotor speed, but not on a symbol-digit paired-associate learning task or an embedded figures perceptual test. The 1- to 3-year abstinent group fell between the two extreme groups on all of the tests.

In our laboratories (Fabian and Parsons 1983), we found that female alcoholics abstinent for 4 years performed significantly better than alcoholics abstinent for 1 month; on individual tests, they scored at, or nearly at, the level of the controls. Nevertheless, when we compared the long-term abstinent alcoholics with the controls on an overall measure of impairment, they scored significantly lower, although the actual difference was relatively small. Compared to the 1-month sober group, the long-term abstinent group scored significantly higher. The conclusion from these cross-sectional studies is that improvement does occur with continued sobriety, but the process is a slow one, extending over a 5-year period for many alcoholics.

One problem with the cross-sectional design is that the independent groups may differ on characteristics other than the usually controlled variables of age and education. Test-retest studies of the same groups avoid that problem, since the same group is followed over time. These studies are very costly and time consuming, and, consequently, there are only a few with a year or more between test and retest. We have conducted three such experiments. In our first study (Fabian and Parsons 1983), at both the test and the retest, the alcoholics differed significantly from the controls on seven of eight tests. Both groups, however, improved on retest. In our second study (Yohman et al. 1985), we tested male alcoholics after 7 weeks of sobriety, to ensure that we were not picking up protracted withdrawal symptoms, and retested them 13 months later on the same battery of neuropsychological tests. Alcoholics differed from controls significantly at both testing and retesting. Again, both alcoholics and controls improved significantly on retest, but they remained as different as at the initial testing.

One other interesting finding present in both studies was that those alcoholics who had resumed drinking during the 13-month study (resumers) had lower levels of performance at the initial test as well as the retest, as compared to those alcoholics who remained sober.

In our third investigation of recovery (Parsons et al. 1990*a*), we retested 73 percent of the alcoholic and control subjects run in the Parsons et al. (1990*b*) experiment described earlier in this chapter. The subjects were retested on the same battery of tests an average of 14 months later. The alcoholics were subdivided into two groups. Resumers were defined as those individuals who were drinking during the 6-month period before retesting (although they were drinking an average of less than one-quarter of their pretreatment intake of alcohol). Abstainers were

defined as those who had remained sober during that period. An overall performance measure was calculated by transforming raw scores on the tests to *T* scores based on the distributions of the entire group at the initial testing. To determine improvement, the retest raw scores on the 11 tests were placed into the regression equations used to derive the *T* scores at the initial testing. Table 5 presents the means of the overall performance score for the three groups at test and retest.

The initial testing conducted at the end of treatment indicated that alcoholics (both those who will become resumers and those who become abstainers) differed significantly from the controls. Those who resumed drinking performed poorer at the initial test than those who abstained. At retest, all groups improved significantly. However, the same significant differences among groups were obtained as in the initial test.

These findings raise some interesting questions. Are the resumers those alcoholics who have lower thresholds for developing alcohol-induced brain dysfunctional states or are they alcoholics who have premorbid cognitive deficits from some yet-to-be identified variables or combination of variables? The answer to this question must come from longitudinal studies starting in the adolescent years.

In answer to the question posed at the beginning of this section, according to the cross-sectional groups analyses, there is evidence for major improvement over a 5-year span of time. Unfortunately, we do not have any adequately controlled longitudinal studies (i.e., which have retested *both* alcoholics and controls) that have lasted more than 13 to 22 months. The controlled studies in the latter range do not find differential improvement between alcoholics and controls. Both groups improve significantly on retest but remain significantly different. Considering both sets of data, it would appear that over the course of 2 to 5 years a considerable improvement occurs, providing the alcoholics do not resume drinking. If this conclusion is correct, then intermediate stage alcoholics' deficits are not permanent. Thus, the premorbid cognitive deficits would appear a minor

TABLE 5

Overall performance: T scores, means, and standard deviations for resumers, abstainers, and controls

Overall performance score

	Initial test	Retest
Resumers (n = 41)	46.1 (7.04)	49.2 (5.35)
Abstainers (n = 62)	49.5 (6.75)	51.9 (6.70)
Nonalcoholics (n = 72)	52.6 (5.73)	55.5 (5.53)

Notes: Standard deviations are in parentheses. A repeated measures ANOVA resulted in a significant group effect [$F(2,172) = 15.0$, $p < 0.0001$], but a nonsignificant group by test-retest interaction [$F(2,172) = 1.0$, $p = 0.371$]. Duncan's multiple range tests for significant ($p < 0.05$) group differences indicated that resumers performed worse than abstainers who performed worse than controls at initial test and retest.

factor, even if present in those who resumed drinking.

Does this improvement result from a reduction in alcoholics' brain dysfunction or is it the product of reorganization of remaining intact areas of brain function? To validate our suggested timeframe for improvement over 2 to 5 years, followup biomedical studies with concurrent neuropsychological testing will be needed and must be conducted for a much longer period than any studies presently in the literature. In any event, the data suggest that the neuropsychological recovery in alcoholics is a long-term process.

These conclusions are similar to those of DeSoto and his colleagues (DeSoto et al. 1985, 1989) based on a much different data set. They used cross-sectional (independent groups) and longitudinal (test-retest) administration of the Symptom Check-List 90 (SCL-90-R) to several hundred male and female alcoholics. The SCL-90-R covers nine primary psychiatric symptom groups, including depression, anxiety, psychoticism, somatization, and hostility. The subjects were Alcoholics Anonymous members who self-reported their years of abstinence. The incidence of psychiatric disturbance, measured by an overall global severity index, dropped sharply from a maximum severity at 3 months to 3.3 years after treatment and then declined more gradually to 7.22 years and then 15.22 years. The latter mean was essentially in the normal range for the general population. They concluded that the keys to recovery are abstinence and time. Remaining abstinent provides the time for "protracted withdrawal syndrome to subside, time for healing of brain dysfunction, time for the repair of social relationships and formation of new ones, time for vocational rehabilitation, time for the rebuilding of lives." (DeSoto et al. 1989, p. 697).

In summary, cross-sectional studies of recovery of neuropsychological functioning in alcoholics suggest that recovery to age-appropriate norms does occur in many alcoholics. It is a long-term process, a process that may be intimately involved in the decrease in psychiatric symptomatology found in DeSoto's studies. Longitudinal studies up to 2 years have indicated that retested alcoholics improve their performance just as much as peer controls, but the groups remain as significantly different as in their original testing. Those alcoholics who will become resumers during the latter part of the year following treatment show greater neuropsychological deficits on the initial testing than those who become abstainers. Whether this results from greater vulnerability to alcohol's toxic effects, another variable, or combination of variables remains to be resolved.

SUMMARY AND CONCLUSIONS

The effects of alcohol-induced brain damage and dysfunction upon cognitive functions are seen most clearly in the alcoholic organic mental disorders, such as alcoholic dementia and the amnestic syndrome (Korsakoff's disorder). Neuropsychological investigations of the latter have found many other cognitive deficits, such as poor abstracting, problem-solving, and visual-spatial perfor-

mance, in addition to the long-recognized severe recent memory disorder.

The intermediate stage alcoholics, constituting about 90 percent of the population of alcoholics, do not have a clinically diagnosable alcohol organic mental disorder. Nevertheless, scores of studies have demonstrated that these alcoholics have poorer neuropsychological performance than peer nonalcoholics (although better than Korsakoff patients) on tests of learning, memory, abstracting, problem-solving, visuospatial and perceptual-motor functioning, and information processing. These deficits range from mild to moderately severe when compared to patients with known brain damage who receive the same tests in the same setting. Recent studies show that alcoholics are less efficient (accuracy divided by time) in performance on all tests. They are not only less accurate, but take considerably longer to complete the tasks.

Not all alcoholics, however, manifest such deficits. In any one study, a range of 50 to 85 percent will display deficits. The following possible determinants of these individual differences were considered: history of alcohol intake variables, family history of alcoholism, childhood behavior disorders, antisocial personality, and, although space precluded discussing them, I can add motivation (Schaeffer et al. 1989) and affective symptomatology (Glenn et al. 1992). For each of these variables, there have been reports of a significant relationship with some aspects of neuropsychological performance. However, none of the relations has been consistently demonstrated. When they are present, they cannot account for the significantly lower performance of alcoholics compared to peer controls. Our conclusion is that prolonged ingestion of alcohol results in brain dysfunctional states that underlie the neuropsychological deficits found in certain vulnerable alcoholics. Identifying the determinants of vulnerability remains a task for the future.

Cross-sectional studies of recovery from neuropsychological deficits in alcoholics suggest that a gradual recovery to, or near to, age-appropriate levels of performance takes place over 4 or 5 years. Longitudinal studies retesting alcoholics *and controls* after 13 to 22 months indicate that both groups differ as much at retest as they did at test, although both groups improve significantly. Obviously, there is a need for a 4- or 5-year test-retest study to clarify the discrepancy between the cross-sectional and longitudinal studies. However, if both sets of findings are valid, the significant gains in recovery apparently occur between 2 and 5 years in sober alcoholics. If so, this process parallels, or perhaps is embedded in, the demonstrated reduction in overall psychiatric symptomatology over a similar period.

The longitudinal studies also have indicated that those alcoholics who will be resumers during the coming year after treatment have significantly poorer neuropsychological performance at time of treatment than those who will become abstainers. Whether this results from a greater vulnerability to the toxic effects of alcohol in the resumers, another variable, or combination of variables, as yet unidentified, remains to be investigated.

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9

SEX DIFFERENCES IN ALCOHOL-INDUCED BRAIN DAMAGE

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INTRODUCTION

National survey data indicate that men are more than four times as likely as women to be classified as heavy drinkers (13 versus 3 percent) and twice as likely to be classified as moderate drinkers (26 versus 13 percent) (Williams et al. 1986). Further studies show that men drink about twice as much as women, both in terms of number of drinks and amount of alcohol consumed. Even after adjustments for sex-related differences in body weight and body water, the average number of milligrams of alcohol consumed daily per deciliter of body water is still 38 percent higher for men than women (Dawson and Archer 1992).

It has been suggested that alcohol consumption has increased disproportionately among women over the past few decades, such that the number of female alcohol abusers is rapidly approaching that of males (e.g., Fraser 1973; Naegele 1979). This suggestion is controversial, however. Wilsnack et al. (1984) reported little overall change in women's drinking patterns over the last few decades. However, they did identify several subgroups of women with increasing consumption and high rates of drinking problems (women who were younger, unemployed, or unmarried, or had a heavy-drinking partner). Fillmore (1987) found a general consistency in women's drinking patterns for 1964, 1967, and 1979, except for a shift toward more frequent heavy consumption in the younger cohorts. Hilton (1987, 1988) found similar patterns of drinking. Ferrence (1980) reported that the perceived increase in female alcoholism rates may be due to several factors, such as (1) an increased social awareness of the particular problems that may face women with alcohol problems (e.g., infertility and fetal alcohol syndrome), and (2) an increase in the proportion of women entering alcoholism treatment programs. In 1987, three-fourths of the 1.4 million Americans treated for alcoholism in the United States were male (NIDA/NIAAA 1989), supporting the current estimates of the male-to-

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female alcoholism ratio at 3–4 to 1 (Grant et al. 1991).

Besides the difference in the rates of alcoholism, sex differences also exist in the age of alcoholism onset, drinking patterns, types and amounts of daily alcohol intake, and years of drinking before treatment. Women tend to begin drinking heavily later in life than men, consume smaller quantities of alcohol, and abuse alcohol for fewer years before seeking treatment (Beckman and Amaro 1986; Parrella and Filstead 1988). Additionally, in national consumption surveys, women's alcohol consumption is evenly divided between beer, wine, and liquor, whereas men consume almost two-thirds of their alcohol intake as beer (Dawson and Archer 1992).

Given these differences in drinking practices and the presence of general biological and psychological differences between the sexes, one might expect to find sex differences in the consequences of chronic alcohol abuse. This chapter will examine alcohol-induced brain damage in male and female alcoholics to assess differences in the incidence, course, and nature of the changes that may vary as a function of sex.

SEX DIFFERENCES IN ALCOHOL-INDUCED NEUROPSYCHIATRIC CONDITIONS

Acute and chronic abuse of alcohol is characterized by both psychological and neurological symptomatology. Following long-term alcohol abuse, a small proportion of alcoholics develop neuropsychiatric disturbances that may be both functionally disabling and permanent. Sex differences are observed in the development and outcome of several of these disorders.

Alcohol Withdrawal Syndrome (AWS)

AWS is characterized by tremor, autonomic arousal, agitation progressing to confusion, and occasionally by hallucinations and seizures lasting from several hours to days after cessation of alcohol intake. Some authors have reported that this disorder is more common in male alcoholics than females; others have reported that the incidence is nearly equal between the sexes. For instance, Gross et al. (1974) stated that men "seem to be at higher risk" for alcohol withdrawal. Lindelius et al. (1974) found that 36 percent of men but only 9.3 percent of women had experienced at least one episode of delirium tremens. Conversely, Sclare (1970) and Wilkinson et al. (1971) found no significant difference between the male and female incidences of delirium tremens. Other laboratories have also failed to find sex differences in reported "shakes," delirium tremens, or alcohol-related seizures (Glenn et al. 1989). Thus, there does not appear to be a clear sex difference with respect to AWS.

Alcoholic Hallucinosis

This syndrome is described as vivid auditory hallucinations following reduction or cessation of alcohol intake. Other signs of withdrawal may also be present, but typically there is no clouding of consciousness. This disorder is reported to be four times more common in males than females (American Psychiatric Association 1987).

Wernicke-Korsakoff Syndrome

This rare but profound disorder associated with chronic alcohol abuse and thiamine deficiency is discussed in detail by Harper and Kril (chapter 3), Oscar-Berman and Hutner (chapter 6), and Cermack (chapter 7). For the purposes of the current discussion, it should be noted that a disproportionately higher number of female alcoholics exhibit this disorder (Victor et al. 1971; Torvik et al. 1982), suggesting that females may be more susceptible than males.

Alcoholic Dementia

Sometimes called "chronic organic brain syndrome," alcoholic dementia is characterized by chronic, progressive failure of memory, loss of intellectual function, and personality deterioration. Ashley et al. (1977) reported that the incidences of dementia were approximately equal for males and females (3.7 percent and 3.0 percent, respectively). However, in a sample of Australian alcoholics, Horvath (1975) found that dementia was approximately three times more common in alcoholic women than men. Horvath also found that the demented subjects (both men and women) were older and had consumed greater amounts of alcohol for more years than their sex-matched, nondemented alcoholic controls. The male demented group had been drinking for a mean of 5 years longer and consumed almost twice as much alcohol on a daily basis than the female demented group. This finding illustrates the greater vulnerability of females to developing dementia with less alcohol exposure than males.

SEX DIFFERENCES IN ALCOHOL-INDUCED STRUCTURAL BRAIN CHANGES

For many years chronic alcoholics have been observed to manifest structural brain changes, both on a microscopic and macroscopic level. These changes were first studied by autopsy, later by pneumoencephalography, and more recently by noninvasive techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) (see Pfefferbaum and Rosenbloom, chapter 4). However, these structural brain changes cannot be interpreted as a direct result of alcohol use. Intervening factors such as age, nutritional status, presence of systemic disease, and history of neurological trauma must also be considered. With respect to sex differences, basic morphological differences between nonalcoholic males and females must be accounted for before conclusions regarding sex-related effects in alcoholism can be drawn.

Autopsy Findings

Harper and Blumbergs (1982) examined the relationship between brain weight (a general marker of brain atrophy), alcoholism, and nutritional status (Wernicke's encephalopathy caused by vitamin B_1 deficiency) and found that male alcoholics with and without nutritional brain damage had significantly lighter brains than normal males. Female alcoholics of both groups also had lighter brains than normals, but the difference did not reach significance. (Females generally have lighter brains than males, so direct male-female comparisons were not conducted.)

The authors concluded that alcohol was as likely as nutritional deficiencies to cause cerebral atrophy and suspected that findings would have been significant in the females if larger numbers of females had been included.

In a second study, Harper et al. (1985) found that alcoholics manifested significantly greater pericerebral space, larger ventricular spaces, and significantly smaller amounts of white matter than controls. The volumes of grey matter and basal ganglia did not differentiate alcoholics from controls, nor did the volumes of cortical hemispheres or frontal regions. Thus, a diffuse, rather than region-specific, loss of white matter associated with alcoholism was supported. Because the authors replicated their original results after the removal of the females from the sample, they concluded that there were no sex-specific effects in their findings.

Additionally, Harper and Kril (1990) reported that male and female alcoholics showed lighter brain weight, greater peripheral space surrounding the brain, and proportionately less white matter than sex-matched controls. Whereas these studies provide scanty information about autopsy findings in females, they provide suggestive evidence that there are few sex differences in the gross brain changes of alcoholism.

In a large Norwegian study of autopsies, Torvik et al. (1982) examined microscopic and macroscopic changes in three alcohol-associated brain lesions: Wernicke's encephalopathy, alcoholic cerebellar atrophy, and diffuse brain atrophy. Wernicke's encephalopathy was divided into active and inactive cases based on the presence of ongoing endothelial cell damage, neuropil destruction, inflammatory response, and perivascular hemorrhage. Cerebellar atrophy was assessed by changes in the anterior and superior parts of the midline vermis. Diffuse atrophy was assessed by gross brain weight. (Females were not included in this measure, so no results are reported here.) Of the cases with suspected or proven alcoholism, 26.8 percent had cerebellar atrophy and 12.5 percent had Wernicke's encephalopathy. The ratio of male to female alcoholics for the sample was 4.2 to 1, which was similar or lower than rates projected for the Norwegian general population. The male-to-female ratio for active Wernicke's encephalopathy, however, was 1.4 to 1, a highly significant deviance from the expected sex ratio. The ratio was corrected to 0.3 to 1 when the sample sex distribution was taken into account. The male-to-female ratios for inactive Wernicke's and cerebellar atrophy were 8.6 to 1 and 6.2 to 1, respectively, but neither ratio represented a significant deviance from expected values when corrected for the sex distribution of the sample. This finding suggested that the disproportionately high number of female cases of active Wernicke's may have been the result of a societal failure to recognize and treat women who abuse alcohol. Males, on the other hand, were more likely to receive treatment (or at least dietary thiamine supplementation) and thus tended to manifest inactive rather than active Wernicke's disorder.

Neuroradiologic Findings

Neuroradiologic findings in chronic alcoholics include enlargement of the ventricular system (particularly the third ventricle) and widening of the cortical and cerebellar sulci with accompanying cerebral and cerebellar atrophy, respectively (e.g., Bergman 1987; Ron 1983; Jacobson 1986*a*). With respect to sex differences, comparisons of CT findings in male and female alcoholics have only recently begun. Much of the early CT work included only male alcoholics (e.g., Ron 1983; Carlen et al. 1984) or presented male and female findings as a single group (e.g., Bergman et al. 1980).

In a sample of male alcoholics, Ron (1982, 1983) reported that, compared with male controls, alcoholics manifested significantly increased ventricle-brain ratios (VBR, an index of ventricle size), enlarged sulci, and increased Sylvian and interhemispheric fissures. Cerebellar sulci were visible only in the alcoholic group, suggesting cerebellar atrophy in addition to the cortical changes. These findings were related to age but not to the duration of alcohol abuse.

In a parallel study of female alcoholics, Jacobson (1986*a*) found that female alcoholics, like males, demonstrated significantly larger VBR, sulci, and Sylvian and interhemispheric fissures than controls. However, female alcoholics did not differ from controls in the size of cerebellar sulci. When the subjects were divided into older and younger subgroups, significant differences emerged between alcoholics and controls in each age range. Younger alcoholics showed a larger degree of ventricular enlargement and Sylvian and interhemispheric fissure widening than did older alcoholics when compared to their age-matched controls. However, the older alcoholics showed a greater degree of sulcal widening. Factors that may have influenced CT findings or the course of alcoholism, such as duration of abuse, history of delirium tremens or withdrawal seizures, amnesic blackouts, and familial alcoholism, all failed to show significant associations with the CT indices.

A more direct test of potential sex differences in the CT findings among alcoholics was conducted by Jacobson (1986*b*), who compared the alcoholics in the Ron and Jacobson studies discussed above. Male and female alcoholics exhibited the same degree of ventricular dilatation and Sylvian and interhemispheric fissure widening. However, males showed significantly greater sulcal widening. The relative lack of significant differences between males and females was especially noteworthy considering the shorter drinking history and lower peak alcohol consumption seen in the female alcoholics. Similar CT results were found when the two groups were statistically equated for consumption variables, body weight, and alcohol-related social complications. Further analyses revealed that age played a role in the sex differences. Younger male alcoholics showed a greater relationship between VBR and sulcal size than their younger controls, whereas older male alcoholics differed little from their controls. Jacobson suggested that these relationships reflected a plateau

effect in which ventricular and sulcal enlargement was manifested early in the male alcoholic career and then leveled out. In contrast, female alcoholics showed the strongest relationship between VBR and sulcal enlargement and age in the *older* alcoholic group. These data suggest that CT changes in females continues to evolve with age and continued alcohol use.

Bergman and colleagues (1980, 1987) have described two types of CT changes that appear in both male and female alcoholics and that apparently vary as a function of age and duration of alcoholism. The first change is a "cortical" change, reflected by dilatation of the cortical sulci. This change has been noted even in young alcoholics with a short duration of the disease. The second change is a "central" change, reflecting dilatation of the ventricular system. Central changes are observed more often in older alcoholics with a longer history of alcohol abuse.

The KARTAD (Karolinska Project Research and Treatment of Alcohol Dependence) project was a large-scale investigation of male and female alcoholics and male and female controls, conducted to examine relationships between CT indices and cognitive performance (Bergman 1987). As in previous studies, Bergman found that both male and female alcoholics displayed significantly greater cortical sulcal width and dilated lateral and third ventricles when compared with their respective controls. (Cerebellar measures were not reported.) When compared with each other, male and female alcoholics did not differ in cortical changes; however, male alcoholics showed a trend toward greater third ventricle dilatation. This pattern most likely reflected basic sex differences, however, since larger third ventricles were also reported in male controls (Bergman et al. 1983).

Female alcoholics demonstrated a consistent trend toward a higher prevalence of neuropsychological impairment than male alcoholics. The authors suggested that these findings reflected a tendency for male alcoholics to show greater morphological changes than neuropsychological changes, whereas female alcoholics had as great, or greater, neuropsychological changes than morphological changes. These results are consistent with the previous conclusion regarding female vulnerability toward accelerated, or greater, alcohol-related cognitive impairment.

SEX DIFFERENCES IN ALCOHOL-INDUCED CENTRAL NERVOUS SYSTEM FUNCTIONAL MEASURES

In assessing the effects of chronic alcoholism on the brain, functional as well as structural processes must be examined. The following section will discuss sex differences in three areas of central nervous system functioning: neuropsychological performance, event-related potentials (electrophysiological functions), and cerebral blood flow.

Neuropsychological Performance

For years, research indicated that the neuropsychological performance of sober alcoholics was impaired relative to nonalcoholic controls (see Parsons, chapter 8).

Briefly, studies have consistently shown deficits in male alcoholics in perceptual-spatial tasks, nonverbal abstracting, perceptual-motor speed, and problem-solving skills (Parsons and Leber 1982; Tarter 1975). Female alcoholics appear to show similar types of deficits, although the number of studies which have systematically examined neuropsychological performance in females has been comparatively smaller than the number of studies with male alcoholics (Acker 1985; Glenn and Parsons 1992; Hatcher et al. 1977; Silberstein and Parsons 1981).

One of the few studies to directly compare the neuropsychological performance of male and female alcoholics was Acker (1986). In alcoholic and control groups equivalent in age, years of education, and a measure of premorbid verbal intelligence, both male and female alcoholics performed significantly poorer than sex-matched controls on a battery of pencil-and-paper tests and automated tests. Male alcoholics performed significantly better than females on only 1 of 27 tests. A similar pattern was seen in the controls, suggesting that the females' lower scores on spatial memory were a nonalcoholic, sex-related effect. The author interpreted the lack of difference between male and female alcoholics as indicating that females showed *greater* alcohol-related impairment. She reasoned that they manifested identical deficits but had drunk heavily for a shorter time and consumed significantly less alcohol than the male alcoholics.

To test this hypothesis, Acker first statistically covaried for the difference in years of heavy drinking. Compared to male alcoholics, females performed significantly poorer on tests of memory, encoding speed, digit span, and spatial orientation. When males and females were individually matched for age and years of heavy drinking, memory, digit span, and psychomotor speed were still significantly poorer for the female alcoholics. The author concluded that these analyses supported the hypothesis of greater alcohol-related neuropsychological impairment in females.

Using the Halstead-Reitan Neuropsychological Battery and the Synonyms and Reasoning test (measures of verbal problem-solving) and the Block Design test (a measure of visual-spatial problem-solving), along with a psychologist's rating of overall intellectual impairment, Bergman (1987) (whose neuroradiological results were discussed previously) found modest to profound neuropsychological impairment in 51 percent of male alcoholics versus 29 percent of male controls and in 63 percent of female alcoholics versus 39 percent of female controls. Bergman reported that the patterns of neuropsychological deficits in male and female alcoholics were similar (the same seven tests showed the greatest magnitude of deficits) but not identical. Relative to controls, the females' deficit was greater than the males, despite the female alcoholics having drunk significantly less alcohol for a significantly shorter period than the males.

In our research laboratory, we have examined the neuropsychological performance of male and female alcoholics using a large variety of measures. Using

summary scores for accuracy and time taken for performance, we reported that both male and female alcoholics took significantly longer than controls to complete the test battery, but only females were significantly different from controls on accuracy scores. Using an efficiency ratio (accuracy/time), males and females were equally impaired (Glenn and Parsons 1990). Using the same sample in a separate investigation, we found that male and female alcoholics performed similarly on measures of verbal and nonverbal memory and learning (Glenn et al. 1988), although the males had been drinking for a longer period than the females.

In a separate sample, we also found that male and female alcoholics scored significantly lower than controls on time, accuracy, and efficiency measures in four areas of neuropsychological functioning: verbal skills, problemsolving, learning and memory, and perceptual-motor tasks (Glenn and Parsons 1992). The alcoholic groups did not show sex differences in their performance. Again, the males had drunk for a significantly longer period than the females (13 years versus 10 years), but males and females did not differ in an index of consumption that combines frequency and quantity estimates.

In a study of alcoholics diagnosed with Korsakoff's syndrome, Jacobson and Lishman (1987) examined male and female performance on tests of memory and global functioning. Female alcoholics showed significantly greater impairment on an index of general intellectual decline and showed greater memory impairment than males. Twenty-three percent of the females showed marked global impairment without marked memory impairment, compared with only 4 percent of the males. The authors reported that this finding was consistent with Cutting (1978), who also found a preponderance of female subjects in their Korsakoff's patients with global involvement. They suggested that caution be exercised in the diagnosis of Korsakoff's syndrome versus alcoholic dementia in female alcoholics.

Event-Related Potentials

The study of the brain's electrical response to external stimuli, i.e., event-related potentials (ERPs), has also been used to evaluate alcohol-induced brain damage (Porjesz and Begleiter, chapter 5). The ERP represents the detection, evaluation, and processing of a stimulus and yields a waveform with several consistent peaks and troughs that may be subject to statistical analysis. The information gained from ERPs appears to differ from the information gained from structural measurements of the nervous system (i.e., CT scans) or from performance measures such as neuropsychological tests.

In describing the ERP deficits most often seen in alcoholics, Williams (1987, p. 123) wrote ". . . deviations in the N100, N200 and P300 are consistent with impairment of brain functions that support selective attention to relevant information sources and that mediate perceptual discrimination and signal analysis . . . ." Specifically, these deviations include decreased amplitudes and/or increased latencies of the N100, N200, and P300 components, when alcoholics are com-

pared with controls. Most of the research in the area of ERPs and alcoholism has centered on the differences between male alcoholics and controls and, more recently, between those who are at risk for developing alcoholism and those who are not. Unfortunately for the study of sex differences, few studies have been conducted using female subjects. To our knowledge, systematic investigation of potential sex-related differences in ERPs in alcoholics has not been conducted outside our laboratory.

Parsons et al. (1990*b*) compared male and female family history positive (FH+) and family history negative (FH-) alcoholics with male and female FH+ and FH- controls on ERPs and neuropsychological performance. The ERPs were assessed using an "oddball" task in which the subject was presented with random sequences of auditory stimuli (high- or low-pitched tones) and visual stimuli (red or green circles) and instructed to keep a mental count of one type of stimulus. Proper identification of a target stimulus by a subject was typically reflected in the ERP waveform by an increase in the amplitude of N100 (a measure of channel or modality selection) and in the amplitude of P300 (a measure of recognition of a stimulus as novel, task-relevant, and requiring further cognitive processing). In other studies, increased latency of parts of the ERP waveform correlated with the degree of cognitive deficit in dementia and Parkinsonism (Polich et al. 1986; O'Donnell et al. 1987).

Parsons et al. (1990*b*) found that alcoholics manifested significantly lower N100 and P300 amplitudes than controls. Comparing males and females separately with their sex-matched control groups, no significant differences for females were found between alcoholics and controls or between FH+ and FH- groups on any of the ERP measures. In males, alcoholics showed significantly smaller amplitudes for N100, Nd (an amplitude difference score), and P300 and significantly longer latency for N200 than controls. The males also showed significant effects of family history as well, with FH+ groups manifesting significantly lower amplitudes at N100 and Nd than FH- groups. Unexpectedly, the FH+ groups also had significantly shorter latency at N200.

On the neuropsychological measures, alcoholics performed significantly poorer than controls on factors of verbal, visual-spatial, perceptual-motor, and semantic memory functions. Females overall performed better than males on the perceptual-motor cluster; however, the difference between alcoholics and controls was greater in the females than in the males. In addition, perceptual-motor tests correlated with the P300 amplitude for both male and female alcoholics.

The results of this study are interesting in light of the findings of significant neuropsychological impairment in both male and female alcoholics (greater impairment in females) but ERP impairment in male alcoholics only. Moreover, the same correlational relationships prevailed between ERPs and perceptual-motor neuropsychological performance in male and female controls.

The authors considered the possibility that the failure to find significant

differences between female alcoholics and controls was related to the shorter history of alcoholism in the females. Dividing the females into groups with short and long histories of alcoholism (the long history group equivalent to the males' history), the authors failed to obtain any significant ERP differences.

In addition, the authors considered the possibility that variations in the menstrual cycle may have led to greater variability among female subjects. However, two recent reports could find no consistent relationship between ERPs and the menstrual cycle (Fagan and Church 1986; Fleck and Polich 1988). No greater variability was noted in the female ERPs relative to those of the males. At this point, the results remain puzzling and await further investigation and replication before final conclusions can be drawn regarding sex differences and alcoholic ERPs.

In a second study from our laboratory, Sinha et al. (1992) examined the test-retest correlation for ERP measures over 14 months. They found that controls had significantly higher test-retest correlations than alcoholics on only 1 of 28 comparisons (N2 amplitude at Oz). Examining sex differences within each group, male and female controls demonstrated comparable correlations on all measures. Female alcoholics had significantly smaller test-retest correlations than male alcoholics for only one measure (P3 amplitude at Pz). These authors concluded that the long-term reproducibility of ERPs over time was generally quite good and was relatively unaffected by alcohol history or sex.

In a third study, we compared ERP measures for alcoholics and controls to determine which ERP measures, if any, would predict at initial testing those alcoholics most likely to resume drinking by the time of retest (Glenn et al. 1993). Although several ERP measures were successful at differentiating future resumers from abstainers (particularly N200 latency), there were no differential effects as a function of sex.

Cerebral Blood Flow

Because of the documented relationship between cerebral functioning, metabolism, and blood flow, measures of cerebral blood flow (CBF) have been used as an indicator of neuronal function (Risberg and Berglund 1987). Abnormalities in CBF have been found in alcoholism and are thought to be caused by the adaptation of the flow to the changed demand for oxygen and glucose by the cerebral tissue. Decreases in CBF have been reported in Korsakoff's patients (Shimojyo et al. 1967), patients suffering from alcoholic dementia (Kruger and Hoyer 1979), and chronic alcoholics who display neither of these disorders (Berglund et al. 1987).

A non-alcohol-related sex difference has been reported with respect to CBF. Females show significantly greater CBF than males in the 40- to 60-year age groups. This difference has been attributed to early sex differences in progesterone levels, oxygen-carrying capacity, and blood viscosity associated with menstruation (Shaw 1987), but becomes insignificant with age. To date, studies with alcoholics have not adequately sam-

pled female subjects and have not controlled for this nonalcoholic CBF sex difference.

There is some suggestion, however, that acute alcohol intoxication is related to acute disruptions in CBF (i.e., stroke) and that the effect may be slightly greater in females than males. In patients under the age of 40, Hillbom and Kaste (1978) reported that alcohol intoxication (consumption of at least 80 g of alcohol within several hours) preceded symptoms of ischemic brain infarction in 20 percent. The risk of stroke with alcohol intoxication was increased by two to three times for men and three to four times for women, suggesting a slight female vulnerability to CBF disruption with intoxication.

SEX DIFFERENCES IN THE REVERSIBILITY OF STRUCTURAL AND FUNCTIONAL CHANGES

The potential reversibility of alcohol-induced deficits is an exciting topic that has relevance for (1) enhancing our understanding of the mechanisms and nature of the initial insult caused by alcoholism, (2) providing recovering alcoholics with realistic predictions for future goals, and (3) improving our understanding of the plasticity of the adult nervous system. A number of studies have reported some degree of change with abstinence, both in the extent of the structural abnormality (e.g., CT indices) and in the amount of functional impairment (e.g., neuropsychological performance). Few, however, have carefully examined sex-related differences in recoverability. The following section will briefly discuss several studies that may facilitate our understanding of these issues (also see Parsons, chapter 8).

Studies at autopsy of structural brain changes in alcoholics provide little insight into the potential for recovery with abstinence. With the advent of CT scans, however, tangible evidence for a return to structural normalcy has begun to accumulate. Ron (1983) found a tendency toward normalization in the width of sulci and Sylvian fissures in alcoholics abstinent for 30 to 152 weeks. Trends toward reversal in cerebral atrophy have also been reported by Carlen et al. (1984) and Cala et al. (1983). This reversibility has led some researchers to question the use of the term "atrophy," preferring instead the term "shrinkage" to better characterize the potentially reversible nature of the cerebral changes (Artmann et al. 1981).

Jacobson (1986*a*) compared recently detoxified alcoholics, AA members (sober an average of 5 years), and controls to assess changes in CT scans with abstinence. (The differences between detoxified alcoholics and controls were discussed above). Male AA members' CT scans were intermediate between alcoholics and controls, but only the cortical sulci widening was significantly less than that of current alcoholics. AA members were not significantly different from male controls. Female AA members also showed intermediate CT indices with no significant CT differences between AA members and controls. However, there were significant differences between current alcoholics and AA members in

ventricle-brain ratio (VBR) and Sylvian fissure widening. The reduction of VBR (taken as a measure of decreasing ventricle size) between alcoholics and AA members was 33.1 percent for females and 15 percent for males, with a reported duration of abstinence in female AA members half that of their male counterparts. Although females were more vulnerable than males to both CT and cognitive alcohol-related deficits, the author concluded that females exhibited superior recovery with abstinence.

In separate studies of male and female alcoholics, modest improvement in neuropsychological functions over 13 to 22 months has been documented (Fabian and Parsons 1983; Yohman et al. 1985). When the improvement in scores of alcoholics was compared with those of controls, however, it appeared that the increments of improvement were parallel. That is, there was no differential improvement for the alcoholics, and the improvement could be primarily attributed to practice effects rather than to recovery.

When alcoholics were divided into abstainers and resumers based on their intertest drinking patterns, both male and female abstainers tended to show better performance on initial testing and greater improvement at followup than those who resumed drinking (Parsons 1987). We found similar results when male and female alcoholics were directly compared using a test-retest design; resumers performed poorer than abstainers, and abstainers were worse than controls at both initial and followup testing. Among the three groups, there was parallel rather than differential improvement. In addition, no significant sex differences were found, indicating that males and females followed similar courses of neuropsychological performance over time (Parsons et al. 1990*a*).

SUMMARY AND CONCLUSIONS

The purpose of this chapter was to examine sex differences in the type and extent of alcohol-induced brain damage. Before reviewing specific findings, it is important to note that sex differences in patterns of alcohol consumption and in duration of abuse were pervasive findings in the studies reviewed. Female alcoholics consumed significantly smaller amounts of alcohol for fewer years in almost every investigation. Despite this, females appeared to show equivalent, if not greater, adverse consequences than their male counterparts.

Neuropsychiatric conditions of alcoholic hallucinosis were more prevalent in male alcoholics than in female alcoholics, whereas females showed an increased risk for development of Wernicke-Korsakoff syndrome and alcoholic dementia. No consistent sex differences were found in the occurrence of alcohol withdrawal syndromes.

Structural brain changes associated with alcoholism included lighter brain weight, ventricular enlargement, and cortical and cerebellar atrophy. These findings were consistent for alcoholics when compared with controls. Although no significant, consistent sex differences overall were found between male and female alcoholics, the studies revealed age differences.

Younger males and older females showed the greatest changes in CT indices. This finding suggests that older males reach a plateau with age, whereas older females show continued progression of changes (Jacobson 1986*b*).

Neuropsychological performance in both male and female alcoholics was characterized by deficits in learning and memory, perceptual-motor speed, verbal and nonverbal abstracting and problem-solving, visuoperceptual analysis, and efficiency. A fairly consistent trend was seen across studies with females performing as poorly as, or more poorly than, male alcoholics despite shorter drinking histories. Among neuropsychiatric patients, female Korsakoff's patients were more likely to display global cognitive impairment as well as marked memory impairment than males (Jacobson and Lishman 1987).

Male alcoholics manifested ERP deficits, with decreased amplitudes and increased latencies on several components. Female alcoholics failed to show any ERP deficits compared with controls, but showed similar correlations between P300 and perceptual-motor functions as males did. Significant sex differences were not found for ERP reliability measures.

Nonalcoholic females showed a biologic tendency toward greater rates of CBF than males. Females who abused alcohol, however, were found to have a slightly increased risk of disruption of blood flow or stroke than males. In recovery from alcohol-induced brain damage, females demonstrated a tendency toward faster and more thorough recovery in structural changes, whereas no sex differences were found in the rate of recovery or in patterns of neuropsychological functions.

"Telescoping" is a popular term used to describe the apparently accelerated course of symptom severity that females suffer. They become more prone to physical complications as well as psychosocial difficulties after a shorter period of alcohol abuse than their male alcoholic counterparts. The evidence presented in this chapter suggests that this term may be aptly applied to structural and functional brain changes as well.

Explanations concerning the vulnerability of females to developing adverse consequences of alcoholism at a greater rate than males have been helpful but not fully adequate to date. Alcoholic liver disease develops more readily in women than in men, and women are also more likely to develop multiple organ damage (Ashley et al. 1977; Morgan and Sherlock 1977; Norton et al. 1987), suggesting a greater female biological sensitivity to alcohol toxicity. In addition, women may be more likely to fast or consume vitamin-deficient diets. These factors would decrease the activity of alcohol dehydrogenase (ADH, the alcohol metabolizing enzyme) and predispose them to physical illness and immunological and neurological disease (US DHHS 1990).

Women have higher blood alcohol concentrations than men after equivalent oral doses (Jones and Jones 1976). One explanation for this is that women's smaller volume of total body water contributes to the increase (Marshall et al. 1983). Recent studies, however, have suggested a second mechanism. These studies have

shown lower levels of gastric ADH and a virtual absence of alcohol metabolism via gastrointestinal mechanisms in females (Frezza et al. 1990). Thus, increased alcohol bioavailability may be the result of alcohol metabolism being largely confined to hepatic processes.

Female alcoholics are also prone to greater psychiatric problems and are more likely to suffer from anxiety and depression (e.g., Shuckit and Duby 1983), factors that may exacerbate existing physical disorders and impair coping mechanisms. Women are significantly less likely to seek advice or counseling from medical, legal, or familial sources (Saunders et al. 1985) and may, therefore, experience greater complications before receiving attention and assistance for their drinking-related problems.

No single factor leading to the increased vulnerability of females to alcohol-induced consequences is likely to be uncovered. An explanation that combines biological, psychological, and social factors will no doubt provide the best approach to understanding this complex and intriguing issue.

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10

APPLICATION OF THEORETICAL MODELS TO THE STUDY OF ALCOHOL-INDUCED BRAIN DAMAGE

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INTRODUCTION

Most of the preceding chapters focus either on identifying specific brain areas or functions that are compromised with chronic alcohol abuse or on clarifying individual differences in these effects. This chapter is included to challenge the reader to consider possible theoretical modes that might be employed to explain these deficits. Other contributors touch on these issues (e.g., Oscar-Berman and Hutner, chapter 6; Cermak, chapter 7; Parsons, chapter 8). However, this chapter provides a more comprehensive and critical examination of the use of theoretical models.

Within this chapter, Toulmin's (1961) statement that "the most appropriate goal of science has always been, and always will be, to give us understanding of the mechanisms involved in the operation of the world and all in it" is accepted. Furthermore, understanding is assumed to entail the description, explanation, and prediction of events and the relations between events.

Description and explanation need no further definition. However, prediction merits additional discussion. Prediction refers to two separate capacities (Carnap 1988). The first is the ability to advance a priori the nature of the relation between nonobservable constructs. In this capacity, prediction relies on the accurate formulation of laws that are founded on the relations between nonobservable (i.e., theoretical) constructs. To achieve this objective, scientists must utilize nonobservable constructs to build a system of "laws" that result in specific predictions regarding the relation between these constructs. Related to this role is the second and perhaps more important capacity, that of predicting new empirical (i.e., observable) laws (Carnap 1988). Thus, the power of a scientific theory lies not only in its ability to predict the relation of the theoretical constructs, but also in its ability to predict new empirical (observable) laws. This translation from abstract, nonobservable constructs to empirical, observable laws occurs by using

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analogies (e.g., rules of correspondence) (Oppenheimer 1956; Carnap 1988).

Using analogies results in the formulation of models that test the predictions derived from the theory. In fact, because theories are abstract, they can be evaluated only by using models. Therefore, adequate tests of theories must utilize adequate models.

CHARACTERISTICS OF SOUND MODELS

The question now arises, "What constitutes an adequate model?" If the objective is to conduct "good science," then adequate models should meet the standard criteria for such activity.

Consistent with other philosophers and scientists, Kuhn (1988) has identified five criteria for the evaluation of scientific theories. He stated that theories worthy of the adjective "scientific" should demonstrate accuracy, consistency, breadth, parsimony, and productivity. Because the means of evaluating theory is through the testing of models, it is logical that these criteria should be also applied to models.

Accuracy refers to the ability of a model to produce predictions that are reflected in experimental outcomes and observation. Consistency is the degree to which a model is congruent not only within itself, but also with existing models of related phenomena. The breadth of a model is defined by the capacity of the consequences to extend beyond the specific observations, laws, or subtheories it was originally designed to explain. Parsimony refers to a model's simplicity, that is, its ability to provide order to a field of inquiry with only a minimum number of laws or conditions. Others have referred to this characteristic as a part of the esthetics of the theory or model. Finally, the productivity of a model is reflected in its ability to reveal previously unobserved phenomena and suggest new empirical laws.

Popper (1988) contends that the most critical characteristic of a scientific model is that it be refutable. According to this logic, the strength of a scientific model lies in the fact that it must be incompatible with certain possible outcomes. Thus, a model within which all outcomes can be accommodated is not scientific. It may be fully descriptive, adequately explanatory, and even predictive, but if it cannot be disproven, it is not scientific.

In summary, models are derived from theories as means of assessing the validity and application of the theory. These theory-driven models are employed by scientists as tools with which to describe, explain, and predict the nature of the world around us. The scientific merit of such models is determined by their accuracy, consistency, breadth, simplicity, ability to generate heretofore unseen relations and empirical laws, and refutability (Kuhn 1970, 1988; Popper 1988).

Obviously, these criteria are not independent and may, in fact, conflict with each other. For example, one model may have superior accuracy, another greater scope. Although accuracy is frequently regarded as the most decisive criteria (excluding refutability), it is, as noted by Kuhn, seldom or never a sufficient criterion for theory or model selection.

EXAMINING THE CURRENT NEUROPSYCHOLOGICAL MODELS

Having reviewed the characteristics of scientific models, the questions of whether our current models meet these criteria and whether one model is superior to another can be addressed. The neuropsychological models proposed to account for the cognitive deficits observed in chronic alcoholics are described and discussed below.[2]

These neuropsychological models are variations on the theme of localization of function. These models are the right hemisphere dysfunction hypothesis, the frontal lobe (frontal–limbic–diencephalic system) hypothesis, and the mild generalized brain dysfunction hypothesis (Parsons and Leber 1981; Oscar-Berman 1987; Tarter 1976). It should be noted that none of the models was presumed to explain all of the empirical data under any or all circumstances. Rather, each was proposed to address particular aspects of the data.

Right Hemisphere Dysfunction Hypothesis

The right hemisphere dysfunction hypothesis postulates that the right hemisphere is differentially sensitive to the neurotoxic effects of chronic alcohol abuse (Oscar-Berman 1987). It was proposed in response to data suggesting that cognitive functions putatively under right hemisphere control (e.g., visual–spatial skills, perceptual–motor skills) are frequently impaired in chronic alcoholics, whereas left hemisphere functions are more often reported to be intact (Parsons and Leber 1981).

Although there is considerable empirical support for this hypothesis (Oscar-Berman 1987; Parsons and Leber 1981), several weaknesses should be noted. First, the model inadequately attempts to *explain* why the right hemisphere should be particularly affected by alcohol abuse. Parsons and Leber (1981) have offered the explanation that ". . . while the toxic effects were undoubtedly pervasive and generalized, the well-rehearsed, practiced, and redundant verbal skills would be more resilient to the toxic effects than the perceptual–spatial, nonverbal holistic skills of the right hemisphere" (p. 331). The problem with this explanation is that it does not address differential sensitivity of the right hemisphere, per se. Instead, it addresses the differential sensitivity of specific cognitive skills that are infrequently rehearsed/employed.

Second, although some studies have failed to obtain significant alcohol-related deficits on verbal (i.e., left hemisphere) tasks, other studies have observed such deficits (Nixon et al. 1987, 1992; Sander et al. 1989; Parsons 1986). Apparently, the distinguishing feature between these two types of studies is the nature of the verbal

[2] *Two models that do not assume underlying brain damage have also been proposed. The first model posits that alcohol-related deficits are the result of negative affective states. The second model hypothesizes that alcoholics' motivational impairment is primarily responsible. Neither of these models has been consistently supported and, therefore, are not discussed here (Nixon et al. 1992; Schaeffer and Parsons 1988; Schaeffer et al. 1989; Mello 1987).*

tasks used. Those studies that indicated verbal deficits were less likely to use tasks involving highly overlearned verbal processes (e.g., Schaeffer and Parsons 1988; Riege 1987; Nixon et al. 1987). These findings suggest that processing in both hemispheres may be measurably impaired if appropriate tasks are utilized.

The right hemisphere hypothesis is testable (i.e., refutable) and can *describe* a subset of the existing data. It is also parsimonious and continues to generate considerable research (e.g., Drake et al. 1990). Internal consistency is quite high because of the parsimonious nature of the hypothesis. Finally, it is also reasonably consistent with theories regarding the nature of cognitive performance in right hemisphere-damaged individuals (Milner 1972; Mountcastle 1962; Sperry 1974).

However, the hypothesis does not meet the criteria of a scientific model. Specifically, it does not accurately describe a significant portion of the literature. Data suggesting left hemisphere damage are not considered in a strict interpretation of the model. Because of its inability to describe the impairment, both explanation and prediction are compromised. Explanation is further compromised due the fact that the hypothesis does not adequately explain why the right hemisphere, per se, should be differentially affected. Given the problems with accuracy, the scope of the model is necessarily compromised.

Frontal Lobe Dysfunction Hypothesis

The frontal lobe dysfunction hypothesis was derived using logic similar to that discussed above. It was proposed in response to the body of data suggesting that frontal lobe functions such as problemsolving and abstraction were frequently impaired in chronic alcoholics. In its original form, the model hypothesized that the frontal lobes, per se, were particularly sensitive to the toxic effects of alcohol (Parsons and Leber 1981). Tarter (1976) has suggested a variant on this hypothesis. In his formulations, the frontal–limbic–diencephalic system is disrupted in chronic alcohol abuse.

The major strengths of the frontal lobe dysfunction hypothesis and its variant are that it is refutable, continues to generate considerable research (Gorenstein 1987; Joyce and Robbins 1991), and is parsimonious. However, it suffers from inadequacies similar to the right hemisphere dysfunction hypothesis. Perhaps most importantly, it does not accurately describe the body of literature. The model accurately describes only that portion of the data that indicates impairment in problemsolving and abstraction processes. It appears that Tarter's variant might improve the descriptive and explanatory powers of the model by integrating limbic structures that are known to affect a wide variety of cognitive functions. However, a substantial body of biomedical and neuropsychological data suggests that neither form of the hypothesis adequately fits the pattern of neuroanatomical and cognitive deficits exhibited by chronic alcoholics (Parsons and Leber 1981; Oscar-Berman 1987). Because description is insufficient, the explanatory and predictive functions are also unmet.

Mild Generalized Brain Dysfunction Hypothesis

The mild generalized brain dysfunction hypothesis proposes that chronic alcohol abuse results in a mild to moderate global dysfunction manifested in a nonspecific and highly variable pattern of cognitive impairment (Oscar-Berman 1987; Oscar-Berman and Ellis 1987). No specific localization of dysfunction is predicted. That is, one alcoholic might demonstrate primarily frontal lobe dysfunction (e.g., abstraction deficits), while another might show right hemisphere dysfunction (e.g., visual–spatial impairment) or global impairment (e.g., poorer scores on several categories of tests). When group data are compiled across a variety of tasks, this intersubject variability produces a pattern of mild global dysfunction.

The strength of the model is its accurate *description* of alcohol-related cognitive impairment. Of the current neuropsychological models, it can best account for the data. The explanatory function is (arguably) better fulfilled than in the previous hypotheses. Because no assumption of differential neuronal sensitivity to the neurotoxic effects of alcohol is made, the explanatory mechanism is defined as the direct effect of alcohol's toxicity on brain cells in individuals who, for whatever reasons, are differentially sensitive. The model is also parsimonious and consistent with other models of the effects of diffuse brain damage on cognitive performance.

Despite these strengths, it suffers considerable flaws as a scientific model. The most significant flaw is that it is not refutable. It can accommodate virtually any observed outcome ranging from no significant impairment to selective impairment of specific cognitive processes. Being untestable, differential predictions cannot be formulated. Therefore, its consistency, in terms of generating new empirical laws, and its scope cannot be evaluated.

In summary, three neuropsychological models have been proposed as scientific models for alcohol-related cognitive deficits. They vary in the degree to which they fulfill the objectives of science and exhibit the characteristics of scientific models. None of the models meet sufficient criteria to be classified as "scientific" according to the Kuhnian principles.

That is not to say that these models are not useful in our attempts to understand the effects of alcohol on cognitive processes. As noted, the mild generalized brain dysfunction hypothesis has proven an accurate descriptor of the group data frequently examined. Furthermore, both the right hemisphere dysfunction hypothesis and the frontal lobe dysfunction hypothesis generate substantial hypothesis-driven research.

The fact that these models are not scientific, according to the Kuhnian perspective, may at least partially reflect the relative immaturity of psychology as a science. Indeed, few (some might say no) models within the field of psychology would meet the stringent criteria for scientific status (Tulving 1987; Gutting 1984).

This failure to achieve scientific status also reflects the focus of neuropsychology as a specialty. Neuropsychology can probably be best described as an "applied" science,

built on the more basic sciences of psychology and neurology. Traditionally, its practitioners have been primarily interested in ascertaining direct brain–behavior links (Tulving 1987; Kolb and Whishaw 1990). They continue to contribute in important ways to our understanding of these links, but they do so largely without reliance on models built from systems of nonobservables (Parsons 1987; Tulving 1987).

Other Models

In recent years, there has been a shift toward using the constructs of cognitive psychology and cognitive science. Dichotomies such as short-term versus long-term memory, declarative versus procedural memory, experiential versus abstractive memory, episodic versus semantic memory, and intentional versus unintentional access, as well as levels of processing approaches, have been used in attempts to clarify the nature of alcohol-related cognitive dysfunction (e.g., Brandt et al. 1983; Squire 1987; Wilkinson and Poulos 1987; Cermak 1977; Graf et al. 1984).

Many, if not most, of these studies have focused on Korsakoff patients and have often used chronic alcoholics as control subjects (Wilkinson and Poulos 1987; Cermak 1977; Graf et al. 1984; Roediger 1990; Cermak, chapter 7). This focus has probably arisen because of the relatively clear dissociation of memory functions in this population and the underlying interest in using this dissociation to clarify memory function in normal populations.

However, there is considerable disagreement over whether the cognitive impairments in Korsakoff patients and chronic alcoholics lie on a continuum or qualify as distinct categories (Parsons et al. 1987; Wilkinson and Poulos 1987). Therefore, a need remains for developing more adequate models to study alcohol-related cognitive deficits in chronic alcoholics who suffer from neither Korsakoff's syndrome nor alcoholic dementia.

COMPONENT PROCESSES MODEL

In light of these facts, Nixon and Parsons (1991; Parsons and Nixon 1993) have proposed a working model adapted from the work of Tariot and Weingartner (1986). As suggested by Kaplan (1988) regarding work with individuals with subtle brain damage, the focus of the theoretical model is on underlying processes instead of endpoint performance measures. Thus, the model is a multiple information store, process-oriented model. Two information stores are proposed in the model. The episodic store involves processes concerned with the learning and memory of context-specific information. This store corresponds to the learning and memory tasks typically utilized in laboratory assessments of learning and memory (e.g., list learning, prose recall, stimulus discrimination, and paired associate tasks).

The second store, the knowledge information store, is associated with processes related to the use of language, logic, meaning, and structural relations. Thus, this store involves those processes related to the use of semantic structures, abstraction-problemsolving, and general information.

It should be noted that there is an inherent asymmetry between the two

stores. The initial acquisition of knowledge information is completed within a specific context (i.e., it is context bound), which is essentially irrelevant to the subsequent retrieval and application of that information. In fact, the knowledge information is used independently from the information regarding the specific episode in which it occurred. For example, although some who are reading this chapter may recall where they were when they heard of the assassination of President Kennedy, recalling that episode is not necessary for retrieval of the general information that President Kennedy was assassinated.

On the other hand, sufficient knowledge information is required for the acquisition of episodic information. For example, subjects in most cognitive tasks must understand or be able to develop an understanding of letters, words, shapes, etc., before they can successfully complete the task (e.g., prose recall). Similarly, subjects required to learn a list of 10 semantically related pairs of words will probably perform better if they can successfully use the semantic structure inherent in the task. Thus, although the acquisition of new episodic information is at least partially dependent on adequate knowledge information, the acquisition of knowledge information is not similarly dependent. That is not to say that episodic memories may not also be formed during the acquisition of knowledge information. Rather, it means that episodic memories are not prerequisites for such learning and subsequent retrieval.

Three component processes are presumed to operate within each store. These processes are availability, access, and efficiency. Traditional definitions of availability and access have been employed (Crowder 1976). Availability refers to the persistence of information over time. Access refers to the ability to retrieve information previously acquired.

Efficiency is often referred to in terms of speed/accuracy tradeoffs (Sternberg 1984). Within the current model, a broader definition is employed. Herein, efficiency refers to the capacity to utilize accurate or relevant information while ignoring or disregarding inaccurate or irrelevant information (Nixon and Parsons 1991).

Within the model, it is reasonable to study both learning and memory processes. Although the processes outlined are typically assumed to be associated with memory processes, evidently, they are also components of demonstrated learning. It might be noted that episodic information lends itself more readily than does knowledge information to the study of learning processes. This situation is reflected in the fact that our work (described below) in knowledge information processes has been limited to protocols in which new information is not required for successful performance.

Alcohol-related cognitive deficits may result from dysfunction in any or all of these component processes within either or both of the information stores. The degree and type of impairment are dependent on the specific process(es) affected and to what extent they are compromised. According to the model, access deficits are characterized by increases in response

time. If only access is affected, accuracy will not be affected.

Impairment in availability is evidenced in increased incorrect responding, which may or may not be accompanied by increases in response time. Obviously, if subjects fail to provide any correct responses, access and availability are hopelessly confounded. Fortunately, this outcome is seldom observed in chronic alcoholics without organic brain dysfunction.

Deficits in efficiency result from an inability to ignore irrelevant or inaccurate information. Therefore, individuals with such deficits are more likely to produce illogical or idiosyncratic protocols (e.g., produce more intrusions), to make inefficient speed–accuracy tradeoffs, and to be unable to accurately isolate relevant responses.

Empirical evaluation of several different aspects of the proposed model is either currently under way or recently completed. Because problems in abstraction and problemsolving are frequently observed in chronic alcoholics, much of our focus has been on knowledge information processes. Therefore, data relevant to these processes will be discussed first.

Knowledge Information

The plant task was first designed as an ecologically valid instrument for assessing cognitive development in the Piagetian framework (Kuhn and Brannock 1977). Since then it has also been used to predict successful completion of alcohol treatment aftercare programs (Erwin and Hunter 1984). Recently the task has been used to assess availability and efficiency processes in knowledge information in male and female alcoholics (Nixon and Parsons 1991).

In this task, subjects are shown four plants. Two of the plants are healthy; two are sickly. The treatment regimen, which each plant receives, is shown and described to each subject. The problem is designed such that only one of the three variables (presence or absence of leaf lotion; size of water glass; type of plant food) is relevant to the plants' outcome (see figure 1).

Following the presentation of the four plants, subjects are told that a fifth, unseen plant is also being cared for. They are also shown the treatment regimen the plant is receiving. Subjects are then asked a series of questions that focus on two objectives: the identification and the isolation of the relevant variable within the experimental protocol.

These two objectives are consistent with the concepts of availability and efficiency within the current model. Specifically, if alcoholic subjects suffer availability deficits, they should be less able to *identify* the relevant variable within the experimental paradigm. Second, if alcoholic subjects exhibit efficiency deficits, they should be less able to *isolate* the relevant variable from the other irrelevant variables within the problem.

The results from Nixon and Parsons (1991) revealed no significant differences between alcoholics and controls in their abilities to *identify* the relevant variable within the experimental context (i.e., the blue plant food). However, alcoholics were significantly inferior in *isolating* the

relevant variable from other variables within the protocol. As one might expect, there were no sex differences on these measures.

These data indicate sufficient information was available to alcoholic subjects to allow them to identify the relevant variable. They were, however, significantly inferior to control subjects in their ability to *isolate* this variable from the irrelevant variables (e.g., size of the water glass) within the problem. From the perspective of the model, alcoholics demonstrated intact availability processes but impaired efficiency processes. Although not conceptualized within the framework of the current theory, other work in our laboratory has also assessed efficiency processes in alcoholics. Glenn and Parsons (1991) utilized the more traditional measure of efficiency, that is, speed–accuracy tradeoffs, in their recent study. These investigators administered a battery of neuropsychological tests that included primarily knowledge information tests to groups of alcoholic and community control females. Subjects completed the tests under one of three instructional sets: speed emphasis, accuracy emphasis, or typical performance instructions. These data indicated that across instructional sets, alcoholics were, as expected, slower and less accurate than controls.

The more interesting aspect of the data was that the two groups responded differently to the tradeoffs necessitated by the instructional sets. Alcoholics were less accurate than controls in the speed emphasis condition but did not differ in accuracy in the other two conditions. On

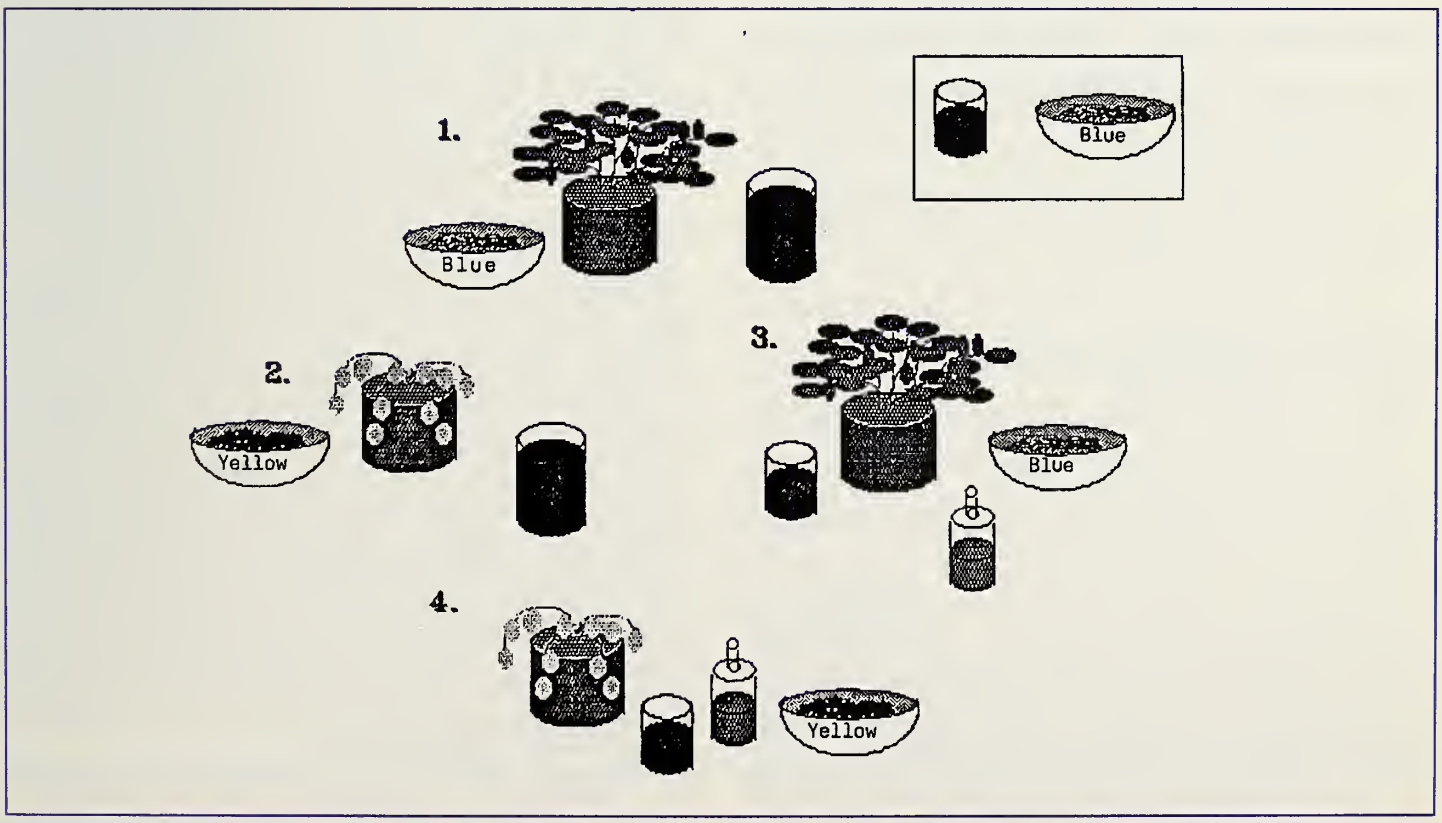

FIGURE 1

Diagrammatic representation of the plant task. In the actual presentation, live plants, real bowls, etc., are used. Plants 1 and 3 are healthy; 2 and 4 are sickly.

the other hand, alcoholics were slower than controls in both the speed emphasis and accuracy emphasis conditions. This performance pattern led the authors to conclude that alcoholics generally appeared to trade speed for accuracy.

Within the proposed model, the direction of the ineffective tradeoff (speed for accuracy or vice versa) is not addressed. The model only posits that efficiency processes may be compromised in alcoholics and that if such impairment exists, it may be observed in a variety of ways, one of which is less effective speed–accuracy tradeoffs.

Additional studies regarding knowledge information function are ongoing. Current protocols include the stem–completion priming paradigm (Graf et al. 1984) to assess unintentional access deficits, a multiple-choice general information test with timed responses to examine intentional access processes, and a difficult verbal fluency protocol to evaluate availability of knowledge information (after Thurstone 1938).

Episodic Learning and Memory

Other studies used a variety of protocols to investigate the fit of the model to episodic learning and memory processes. These protocols include tests of prose acquisition, paired associate learning and recall, and serial learning and recall. These data, although very preliminary, are quite promising. By way of example, only one of the studies will be described.

If episodic information is context bound, a pertinent variable affecting learning and memory for such information should be the environmental context. Although there are exceptions (see Nixon and Kanak 1985), much of the previous work with environmental context manipulations has shown that subjects generally perform better if acquisition and test occur in the same room (i.e., a common environmental context) (Smith 1979; Smith et al. 1978)

In a current study in our laboratory, the environmental context is being explicitly manipulated between study and test phases to determine whether alcoholics' access to information is differentially affected by changes in the implicit cues from the environment. The protocol requires that subjects learn a list of 12 adjective–nonsense trigram pairs to either a low (4/12 trigrams correct) or high (8/12 trigrams correct) degree of learning (DOL). The DOL manipulation was included because previous work had suggested an interaction of context manipulation and DOL (Nixon 1982).

Following the acquisition phase, subjects engage in noninterfering tasks in a neutral context. After a 20-minute interval, subjects return to either the original context or a different context for an unannounced recall test. In the test, subjects are presented with the first letter from the previously learned trigrams and asked to provide the missing two letters in the correct order. Each cue is presented individually, and subjects are allowed 10 seconds to respond to each cue.

Using this protocol, one can examine (1) access deficits by investigating the differential effects of the environmental context manipulation and (2) availability deficits by considering correct responding.

Acquisition and recall data from 45 subjects (24 controls, 21 alcoholics) have undergone preliminary analyses. These data indicate that alcoholics achieved significantly fewer correct responses per trial, regardless of the DOL (4 versus 8 correct). As expected, subjects in the higher DOL required significantly more trials to achieve criterion than those in the lower DOL. There was no interaction between group and DOL.

As anticipated, there was a group main effect in the expected direction (alcoholics performing more poorly) on the number of trigrams correctly completed on the memory test. Additionally, these preliminary data suggested that alcoholics and controls may differ in their response to changes in the environmental context with the interaction of group and test conditions (same versus different). Control subjects actually benefited from testing in a context different from the acquisition context; alcoholics performed worse under this condition.

Overall, these data suggest alcohol-related deficits in both access and availability processes. Access deficits were suggested by the differential response to context changes described above. One possible explanation for this pattern is that control subjects differentially benefited from a release of inter-item interference when in the different context. Availability deficits were demonstrated by the reduced number of trigrams completed correctly by alcoholic subjects.

THE SCIENTIFIC MERIT OF THE PROPOSED MODEL

Although it is obviously premature to attempt to fully evaluate the scientific merits of the component processes model, examining the model at this point may identify strengths as well as potential weaknesses. In considering the three objectives of science, it appears that the model can adequately describe and explain the highly variable findings obtained in alcohol studies.

Regarding prediction, the conclusion is more tentative. Differential predictions regarding cognitive performance can be derived and tested. Thus, the model at least partially fulfills the predictive function. However, the more powerful aspect of prediction, as noted previously, is the ability to predict *new* empirical laws. It is not clear whether the current model will adequately fulfill this function. Additional empirical studies utilizing the model will need to be conducted before this question can be answered.

The degree to which the model demonstrates the five characteristics of scientific models is also difficult to assess at this point in its development. Some general statements regarding these characteristics can be noted, however.

Accuracy

The initial data suggest the model can be used to accurately reflect the empirical data. Deficits in learning, memory, and abstraction-problemsolving processes can

be identified within the framework of the model.

Consistency

The model is both internally and externally consistent. That is, the model provides a cohesive framework for the study of cognitive processes in alcoholic populations and is consistent with current thinking regarding the organization and function of cognition.

Breadth

Although empirical support is not yet available, there is no obvious reason why the model could not be applied to studying cognitive functions in virtually any population in which general information and episodic information can be operationally differentiated. The model might provide a powerful framework for studies integrating animal and human work.

Parsimony

The model is quite parsimonious, utilizing only a few abstract concepts to address the complicated processes generally referred to as learning and memory.

Productivity

This characteristic is the most difficult to assess. Certainly the model can provide direction for programmatic, hypothesis-driven research programs investigating cognitive processes. However, whether the model will have application in other fields is not readily evident.

Overall, the model is promising but needs additional empirical study. Based on the criteria discussed, the model is superior as a "scientific" model relative to the neuropsychological models previously proposed. However, the model is not without insufficiencies. The primary scientific inadequacy of the model is that it may not be easily applied to areas outside those to which it was originally intended. That is, its productivity may be limited. A second problem, not entirely separate from the first, is how the model would invoke a consensus regarding how we, as cognitive neuroscientists, should conduct science.

Because these issues of consensus and productivity are at the center of the debate of scientific merit (Gutting 1984; Kuhn 1970), they demand further discussion. It has been argued that the inability of certain fields of inquiry such as psychology, sociology, and psychiatry to achieve significant consensus regarding questions, protocols, and procedures precludes their research endeavors from being accepted as "empirical science" (Gutting 1984).

Certainly, a consensus is not likely obtained to the same degree within these fields as it is in other fields, such as physics or mathematics. However, it could be argued that imposing the Kuhnian perspective on these areas of study is inappropriate (see Gutting 1984 for a discussion). It might be noted that even Kuhn (1970) stated that the perspective was appropriate to the natural sciences only—that is, science whose subject matter has discoverable objective form. This definition of natural science forces an evaluation of our subject matter.

Historically, much of the subject matter examined by psychologists, psychiatrists, and other "social scientists" has not

yielded itself to a definition requiring a discoverable objective form. However, the continuing development of cognitive neuroscience and the accompanying technology has provided new tools and focus for many investigators of brain–behavior relations. With these advancements, the opportunity to discover objective form is approaching. Therefore, work on brain–behavior interactions will likely be increasingly considered as a natural science.

Of course, even if classified as a natural science, it is not required that these stringent criteria be applied to the study of brain–behavior relations. There are questions that do not demand their application. However, if the opportunity for scientific advancement is to be maximized, applying these criteria will be advantageous.

Recognizing cognitive neuroscience as a natural science does not, however, eliminate concerns regarding consensus. Cognitive neuroscience embraces researchers from areas as diverse as linguistics, psychology, computer science, anthropology, and neurology (Lister and Weingartner 1991; Posner 1989). These researchers ask a variety of questions from a variety of perspectives. It is unlikely that formulation of a single paradigm to guide scientific inquiry will be achieved in the near future. This fact does not, however, preclude research from proceeding scientifically.

Conducting science is a developmental process. Only as a science matures will paradigms in the Kuhnian sense be observed. In the process of maturing, several different "sub"-paradigms or supertheories may coexist (Kuhn 1970). The crucial issue is not whether a single paradigm will eventually be accepted or what form that paradigm might assume. Rather, the critical issue concerns the nature of the research being conducted during the process.

Over the past several decades, work regarding brain–behavior relations has been primarily descriptive. If we are to fully realize Toulmin's concept of science, testable models must be developed and applied to the field. This process of development can be tedious and frustrating; identifying appropriate theoretical constructs and analogies may be initially fruitless. As a maturing science, we must commit ourselves to the process. Oppenheimer (1956) provided fitting insight about where we might start: ". . . all sciences arise from refinements, corrections and adaptations of common sense" (p. 128).

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STRUCTURAL AND FUNCTIONAL CHANGES IN ANIMAL MODELS

11

ALTERATIONS IN THE STRUCTURE OF THE HIPPOCAMPUS AFTER LONG-TERM ETHANOL ADMINISTRATION

Don W. Walker, Ph.D.,[1,2] Michael A. King, Ph.D.,[1,2] and Bruce E. Hunter, Ph.D.[1]

INTRODUCTION

Given the difficulties in identifying specific structural and functional damage in humans chronically exposed to ethanol, animal studies, which provide considerably more control, are essential components of ethanol research. During the past two decades, controlled studies using laboratory animals have provided convincing evidence for the specificity of chronic ethanol exposure in inducing abnormalities of brain structure and function. However, neither the mechanism(s) of ethanol neurotoxicity nor the specific morphological or functional basis of ethanol-induced cognitive deficits has been established. Nevertheless, we have learned much about the structural and functional abnormalities induced by chronic ethanol as previously reviewed (Walker et al. 1980*a*, 1981, 1982; Walker and Hunter 1987). This chapter will be limited to studies of the effect of chronic ethanol exposure on the structure of the hippocampus in mature rodents. The effect of gestational or early postnatal ethanol treatment on the hippocampus has been recently reviewed (Jones 1988; West and Ward 1992) and will not be considered in this article.

METHODOLOGICAL CONSIDERATIONS

Chronic ethanol treatment (CET) in animals has been defined liberally by alcohol researchers as extending from as little as 4 to 5 days to as long as 18 months. In this review, CET is the duration of continuous ethanol exposure likely to result in lasting (or permanent) structural and functional alterations of the hippocampus (or its neural connections). These alterations require a minimum of 2 to 4 months of ethanol exposure to develop. Ethanol treatment for only a few days can induce tolerance and/or physical dependence but produces a disturbance of neuronal function thought to last for only a few days.

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Although neuronal damage associated with prolonged exposure can occur at levels of ethanol exposure insufficient to produce overt evidence of physical dependence (Walker and Freund 1971; Walker et al. 1981; Walker and Hunter 1987), apparently both ethanol neurotoxicity and physical dependence can occur simultaneously. In studying the brain damage associated with prolonged CET, a sufficient ethanol-free period posttreatment is thus desirable so that the lasting functional disturbances associated with structural changes can be separated from the transient disturbances associated with the ethanol withdrawal reaction.

A critical evaluation of the existing literature concerning the effect of CET on hippocampal structure must include the method of ethanol administration and the control of nutritional factors. To achieve satisfactory nutritional control, a control group must be included that is nutritionally yoked to the CET group by pair-feeding procedures. In this way, the animals in the CET and control groups ingest the same total calories and the same amount of vitamins and minerals. This is necessary because the so-called "empty calories" provided by ethanol will displace nutrient-containing calories in regular laboratory food, and the ethanol-consuming animals will eat less laboratory food and correspondingly ingest lower amounts of vitamins and minerals. In all of the studies from our laboratory, the experimental animals (mice or rats) are maintained on nutritionally complete liquid diets containing 8- to 10-percent v/v ethanol in which ethanol comprises 35 to 40 percent of the available calories (Walker and Freund 1971; Walker et al. 1980*b*). Control animals are pair-fed an equivalent diet in which sucrose is substituted isocalorically for ethanol or receive unlimited access to dry laboratory food and water. This method of ethanol administration results in an average daily intake of 12 to 14 g/kg in rats and 20 to 25 g/kg in mice resulting in a morning blood ethanol concentration of 100 to 200 mg/dL (Orona et al. 1988; Rachamin et al. 1989). Most laboratories investigating the effect of CET on the structure of the hippocampus now use methods of ethanol administration that include pair-fed nutritional control groups.

BEHAVIORAL MANIFESTATIONS OF ETHANOL NEUROTOXICITY

Lasting behavioral deficits can serve as a sensitive indicant of ethanol neurotoxicity and permit insight into the underlying structural and physiological aberrations that underlie such functional disturbances. Many studies during the past 20 years have reported lasting behavioral deficits following CET in rodents under nutritionally controlled conditions. These experiments have demonstrated that CET results in a progressive decline in learning and memory, as assessed by a variety of behavioral testing paradigms including active shock avoidance (Freund and Walker 1971; Triet et al. 1980; Walker and Freund 1971), complex maze learning (Bond and DiGiusto 1976; Fehr et al. 1976), and tests of temporal (Denoble and Begleiter 1979; MacDonall and Marcucella 1978; Walker and Freund 1973; Walker

and Hunter 1978) and spatial memory (Arendt et al. 1988*a*, 1989; Beracochea and Jaffard 1985; Beracochea et al. 1987; Hodges et al. 1991; Walker et al. 1981). In general, the learning and memory deficits are progressively more severe as the duration of CET is increased and are apparently irreversible following CET cessation of sufficient duration (Freund and Walker 1971; Arendt et al. 1989). The specific morphological or functional basis of the ethanol-induced memory deficits is not known. However, evidence has begun to accumulate for a role of the hippocampus and/or its afferent innervation from the basal forebrain in certain types of memory (see Arendt, chapter 22).

THE HIPPOCAMPUS AS A MODEL SYSTEM TO INVESTIGATE THE NEUROTOXICITY OF CHRONIC ETHANOL EXPOSURE

The hippocampal formation has been a major model system used to study the structural and functional consequences of the neurotoxicity produced by CET for several reasons including (1) the hippocampus plays a putative role in memory; (2) the vast available information on the normal anatomy, physiology, and pharmacology of the hippocampus provides a significant and useful database; and (3) the relatively simple and highly laminated structure of the hippocampal formation simplifies quantitative morphological and physiological measurement, thereby enhancing detection of possibly subtle alterations produced by CET. Because the hippocampal formation is the brain structure serving as the model system for the work to be discussed below, a brief description of the basic anatomical features is provided.

Basic Anatomy of the Hippocampal Formation

Figure 1 is a schematic transverse section illustrating the organization, major divisions, and principal cell types of the hippocampal formation. The hippocampal complex is divided into two regions: the hippocampus [Ammon's horn or cornu ammonis (CA)] and the dentate gyrus (DG). The intrinsic synaptic organization of the hippocampus is marked by several salient characteristics. First, an intrinsic trisynaptic series of excitatory connections link the entorhinal cortex to the DG, the DG to CA3, and finally CA3 to CA1. Second, the CA and DG are arranged in a laminar fashion and are trilayered, each composed of a tightly packed, principal cell layer, bordered by layered, cell-poor neuropil. Third, the specific afferent terminations of the hippocampus are also laminated, so that each class of afferents terminates upon relatively specific portions of the dendrites of CA and DG cells. Finally, the lamellar organization of the hippocampus is such that a slice transverse to the longitudinal axis of the hippocampus 300 to 500 μM thick preserves the basic trisynaptic series of intrinsic connections.

The hippocampal formation receives a major afferent projection via the fornix from the medial septal nucleus (MS) and the vertical limb of the nucleus of the diagonal band (VDB). This septohippocampal projection provides more than

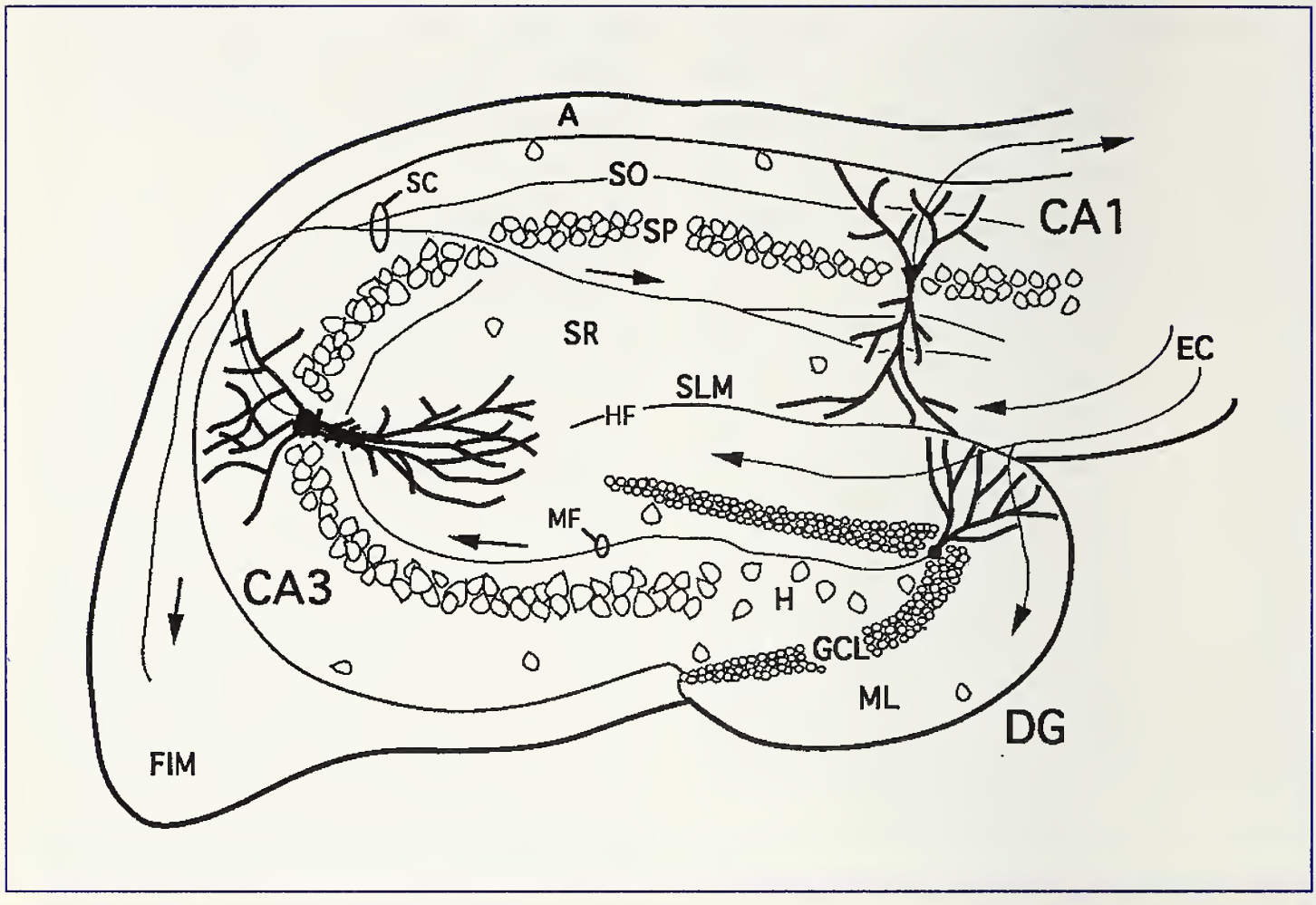

FIGURE 1

A schematic transverse section through the hippocampal formation illustrating the divisions, organization, and principal cell types. Abbreviations: A, alveus; CA, cornu ammonis; DG, dentate gyrus; EC, entorhinal cortex; FIM, fimbria; GLC, granule cell layer; H, hilus; HF, hippocampal fissure; MF, mossy fibers; ML, molecular layer; SC, Schaffer collaterals; SLM, stratum lacunosum moleculare; SO, stratum oriens; SP, stratum pyramidale (pyramidal cell layer); SR, stratum radiatum.

90 percent of the cholinergic innervation of the hippocampus (Amaral and Kurz 1985; Mesulam et al. 1983).

Hippocampus and Memory

There is a growing recognition that the hippocampus has an essential role in certain types of memory (Milner 1959; Olton et al. 1979; Squire 1986; Zola-Morgan and Squire 1990). Research in both humans and animals supports the hypothesis that projections of the cholinergic basal forebrain neurons from the MS/VDB to the hippocampus and from nucleus basalis to the neocortex play important roles in memory function (Bartus et al. 1982; Coyle et al. 1983; Squire 1986). The projections from MS/VDB to the hippocampus (septohippocampal pathway) appear to be particularly important for memory (Perry et al. 1977; Gage et al. 1984; Jaffard et al. 1985; Hagan et al. 1988). Damage to the cells of origin of the septohippocampal pathway (Hagan et al. 1988), destruction of the postsynaptic target cells in the hippocampus (Olton et al. 1979), or severing the axons of the projecting cells in the fimbria-fornix (Olton et al. 1979; Will and Hefti 1985) produce similar deficits in memory, suggesting that the

integrity of each component of the septohippocampal pathway is required.

Some recent studies have provided support for the hypothesis that CET produces a permanent impairment in cholinergic transmission in the septohippocampal pathway, resulting in a disturbance of memory function (Arendt et al. 1989; Beracochea et al. 1986; Hodges et al. 1991). Beracochea et al. (1986) reported that mice exposed to CET for 7 months were severely impaired in a delayed spontaneous alteration test of memory when tested 3 to 9 weeks after CET was discontinued. Parallel analysis showed that CET significantly decreased sodium-dependent, high-affinity choline uptake in the hippocampus. The memory deficit of mice given CET dramatically improved to control levels after administration of physostigmine (an acetylcholinesterase inhibitor) before behavioral testing. These results suggest that the memory deficit induced by CET is related to cholinergic dysfunction.

A recent series of studies provides further support for the hypothesis that memory deficits associated with CET may be mediated by a loss of cholinergic neurotransmission in the septohippocampal system (Arendt et al. 1988*a*,*b*, 1989; Hodges et al. 1991). CET in rats produced a loss of cholinergic neurons in the basal forebrain. This effect paralleled a decrease in several markers of cholinergic function in the basal forebrain and hippocampus, including a decrease in acetylcholine and a reduction in the activity of choline acetyltransferase and acetylcholinesterase (Arendt et al. 1988*a*, 1989; Hodges et al. 1991; also see Arendt, chapter 22). Administration of CET for 4 to 28 weeks produced a progressive decline in spatial and nonspatial memory assessed in a radial arm maze that was paralleled by progressive decreases in markers of cholinergic synaptic function. The deficits in memory and cholinergic function were reversible after 8 weeks of CET, partially reversible after 18 weeks of CET, and irreversible after 28 weeks of CET (Arendt et al. 1989). The loss of cholinergic neurons in basal forebrain observed after 12 weeks of CET were more pronounced in the medial septum and diagonal band regions, which project to the hippocampus, than in the nucleus basalis, which projects to the neocortex (Arendt et al. 1988*b*).

Both the memory deficit and the reduced cholinergic function were substantially reversed by transplants into the hippocampus and/or neocortex of cholinergic-rich fetal cell suspensions but not reversed by noncholinergic transplants (Arendt et al. 1989; Hodges et al. 1991; see also Arendt, chapter 22). Rats given CET for 28 weeks also showed improvements in memory function after treatment with cholinergic agonists and disruption with cholinergic antagonists at low doses not effective in controls. This series of studies may have considerable significance in providing a link between atrophy of hippocampal and basal forebrain neurons, septohippocampal cholinergic dysfunction, and impaired memory resulting from CET. However, additional validation is required, particularly since CET was administered in these studies via

the drinking water without a pair-fed, nutritional control group. In addition, since the effect of CET on hippocampal structure was not evaluated, it is not resolved whether the observed memory and cholinergic dysfunction is attributable to primary neurotoxic effects on basal forebrain cholinergic neurons or on target hippocampal neurons.

Arendt et al. (1988*b*) have assumed that CET produces a direct neurotoxic effect on cholinergic neurons in the basal forebrain that produces a secondary degeneration of axon terminals in cortex and hippocampus. Since cholinergic basal forebrain neurons in the MS/VBD are dependent on the hippocampus for trophic support (Barde 1989; Gage et al. 1988; Hefti 1986; Whittemore and Seiger 1987), an alternative possibility is that the initial site of action of CET is on postsynaptic hippocampal neurons resulting in reduced trophic support, and thus secondary degeneration, of cholinergic septohippocampal neurons (see Walker et al., chapter 20).

NEURONAL LOSS IN THE HIPPOCAMPUS AFTER CHRONIC ETHANOL CONSUMPTION

Few laboratories have investigated the effect of long-term ethanol exposure on the number of neurons in the hippocampal formation. The available evidence, however, strongly suggests that CET results in a 10- to 40-percent loss of hippocampal pyramidal cells (Bengoechea and Gonzalo 1991; Beracochea et al. 1987; Cadete-Leite et al. 1989*b*; Lescaudron and Verna 1985; Phillips and Cragg 1983; Walker et al. 1980*b*, 1981), DG granule cells (Cadete-Leite et al. 1988*a*,*b*; Durand et al. 1989; Walker et al. 1980*b*, 1981), and local circuit interneurons in the hippocampus (Lescaudron et al. 1986) and DG (Scheetz et al. 1987*a*). Several variables have an impact on the extent, time-course, and selective regional vulnerability of neuronal loss in the hippocampus resulting from CET. These variables are considered later in this review.

The first evidence that CET resulted in a loss of neurons in the hippocampal formation was reported by our laboratory about 12 years ago (Walker et al. 1980*b*, 1981). We found that 5 months of CET resulted in a 16-percent loss of pyramidal cells and a 20-percent loss of DG granule cells in the dorsal hippocampal formation, when assessed 60 days after CET was discontinued (Walker et al. 1980*b*). These findings led to the question of whether the time-course of structural changes in the hippocampus paralleled the time-course of behavioral abnormalities produced by CET. Evidence from behavioral studies (Freund and Walker 1971; Walker et al. 1982) had shown that 3 months or more of CET produced progressively more severe behavioral deficits, but no effects were discernable after 6 weeks of CET. We exposed rats to CET for 10, 20, or 30 weeks and assessed the neuronal loss in the hippocampus and DG after a 60-day ethanol-free period (Walker et al. 1981). The number of CA1 pyramidal cells was reduced 16 to 28 percent after all durations of CET, with the 10-week group being least affected. However, neither the number of CA2-4 pyramidal cells nor the

number of DG granule cells was altered significantly by 10 weeks of CET. All three types of neurons were reduced significantly by 20 or 30 weeks of CET. These results support the hypothesis that region CA1 is particularly sensitive to the neurotoxic effects of CET, and that the time-course of neuronal loss during CET compares favorably with the time-course of development of behavioral deficits during CET.

The hypothesis that hippocampal pyramidal neurons may be more sensitive to CET-induced neuronal loss than granule cells of the DG has recently received further support. Bengoechea and Gonzalo (1991) found that 70 days of CET, without withdrawal, resulted in a significant decrease in the number of hippocampal pyramidal cells but not of DG granule cells.

Other evidence suggests that pyramidal cells and interneurons located in the ventral portion of the hippocampus are more sensitive to CET than are those neurons in the dorsal hippocampus (Lescaudron and Verna 1985; Beracochea et al. 1987; Lescaudron et al. 1986). The effects in mice of 9 months of CET, followed by 3 months of withdrawal, on the number of CA1 pyramidal cells in the dorsal and ventral hippocampus were examined (Lescaudron and Verna 1985). Compared with pair-fed control mice, a loss of CA1 pyramidal cells was observed in mice given CET that was greater (–19 percent) in the ventral hippocampus than in the dorsal hippocampus (–8.5 percent).

Beracochea et al. (1987) also studied the effect of administration of CET to mice for 8, 24, or 48 weeks on the number of CA1 pyramidal cells in the dorsal versus ventral hippocampus and on memory as assessed by a spatial alternation task. Eight weeks of CET did not affect memory or neuronal number, whereas 24 or 48 weeks of CET reduced both memory function and neuronal number. CET for 24 weeks significantly reduced the number of CA1 pyramidal cells by 11.4 percent in the ventral hippocampus but not in dorsal hippocampus. The number of CA1 pyramidal cells was reduced by 23 percent in the ventral hippocampus and by 17 percent in the dorsal hippocampus by 48 weeks of CET.

GABAergic interneurons in area CA1 of the ventral hippocampus are apparently also more vulnerable to CET than are such neurons in the dorsal hippocampus. Lescaudron et al. (1986) found that CET for 6 months without an ethanol withdrawal period produced a 25-percent reduction of GABA-immunoreactive interneurons in area CA1 of the ventral hippocampus but not in the dorsal hippocampus. The reason for the apparent increase in vulnerability of the ventral hippocampus to CET is not known. However, the cholinergic innervation of the ventral hippocampus from the MS/VDB in the basal forebrain is proportionally much greater than that observed for the dorsal hippocampus (Dravid and Van Deusen 1984; Yoshida and Oka 1990). The septohippocampal projection neurons might then be more vulnerable to loss of trophic support by damage to the ventral hippocampus than to the dorsal hippocampus.

Another important issue is the potential contribution of ethanol withdrawal

following prolonged CET to the neuronal loss in the hippocampus. Almost all of the studies from our laboratory have included an ethanol-free withdrawal period of at least 60 days before anatomical measurements are made (Walker et al. 1981, 1987). This period of "recovery" has been included so that results from our morphological experiments can be readily compared to parallel experiments examining the effects of CET on functional behavioral or electrophysiological parameters. An ethanol-free withdrawal or recovery period was deemed important to eliminate possible transient effects of ethanol withdrawal on functional measures of ethanol neurotoxicity and to allow assessment of possible recovery of function.

Phillips and Cragg (1983) exposed mice to CET for 4 months with or without a withdrawal period of 4 months and assessed the effects on the number of CA1 pyramidal cells. The number of these neurons was unchanged by CET without a withdrawal period but was reduced by 9 percent in the group given the 4-month withdrawal period. They suggested that neuronal loss in the hippocampus occurred during the withdrawal period following CET, rather than during ethanol exposure. This observation clearly called for further studies including a comparison of the effects of CET with and without a withdrawal period.

Subsequently, several groups of investigators have reported substantial loss of both pyramidal and granule cells in the hippocampal formation resulting from CET alone without a withdrawal (Bengoechea and Gonzalo 1991; Beracochea et al. 1987; Cadete-Leite et al. 1988*a*,*b*, 1989*a*; Lescaudron and Verna 1985). Interestingly, it has been reported that loss of granule cells in the DG during CET may continue during the withdrawal period (Cadete-Leite et al. 1988*a*). These investigators found that 18 months of CET without withdrawal reduced the number of granule cells by 37 percent, whereas 12 months of CET followed by 6 months of withdrawal reduced the number of granule cells by 50 percent. These results suggest that once the mechanisms underlying CET-induced neuronal death in the DG are triggered, they continue to act even after ethanol exposure is ended. This continued cell loss during withdrawal from CET is apparently limited to the DG granule cell since loss of CA3 pyramidal cells during CET does not continue during withdrawal (Cadete-Leite et al. 1989*a*).

A final issue to be addressed regarding hippocampal neuronal loss induced by CET is the potential role of genetic susceptibility to ethanol neurotoxicity. Most of the studies of this kind conducted in our laboratory and others have used outbred rat strains. We have observed a wide range of responses to CET in individual animals. That is, some animals are dramatically affected by CET, and others are apparently resistant to CET. This differential vulnerability serves to increase statistical variability. One approach has been to study the effect of CET on hippocampal neuronal number in lines of mice that have been selectively bred for their differential susceptibility to the soporific effects of acute ethanol administration. Scheetz

et al. (1987*a*,*b*) compared the effect of 3 months of CET on the frequency of basket cell interneurons in the DG granule cell layer of short sleep (SS) and long sleep (LS) mice. CET did not affect the ethanol-resistant SS mice, whereas CET produced a 16-percent decrease in basket cell frequency in the LS mice. These results suggest that genetic differences between these two strains might play a role in the sensitivity of an animal to ethanol-induced brain damage. The possibility that the vulnerability to ethanol neurotoxicity is genetically influenced should be pursued further, because such an approach could be valuable in increasing our understanding of the possible mechanisms of ethanol-induced neuronal damage.

MORPHOLOGY AND SYNAPTIC CONNECTIVITY OF HIPPOCAMPAL NEURONS SURVIVING CHRONIC ETHANOL EXPOSURE

Because of the well-known intrinsic and commissural monosynaptic connections of the hippocampal formation (Fricke and Cowan 1978; Laurberg 1979), the neuronal loss induced by CET necessarily results in partial deafferentation of surviving hippocampal and DG neurons. Furthermore, deafferentation in the hippocampus is known to produce changes in dendritic morphology including spine loss and decreased dendritic branching, which recovers with time due to sprouting of undamaged afferents (Cotman and Nadler 1978; Parnavelas et al. 1974). It is likely that CET produces a complex sequence of changes, including destructive cell death, deafferentation, and synaptic reorganization. Neurons surviving CET will likely obtain some new targets for their projections and receive additional afferents to replace those that were lost. It is probable that destructive and compensatory changes occur simultaneously during CET and that further reorganizational changes may occur following CET. Some neurons destined to die may exhibit dendritic dying back and loss of synapses preceding death. On the other hand, other neurons not damaged irreversibly by CET may become hyperinnervated and/or hypertrophied by gaining afferents that have lost contact with dead or dying cells. With these considerations in mind we will address several relevant questions concerning the fate of hippocampal neurons surviving CET including the following: (1) What changes in dendritic morphology are observed in hippocampal neurons surviving CET, and are they reversible? (2) Are there changes in synaptic density in the terminal fields of hippocampal neurons surviving CET? (3) What is the effect of CET on the capacity of surviving hippocampal neurons for reactive synaptogenesis?

Dendritic Morphology of Hippocampal Neurons after CET

It is important to learn if hippocampal neurons that have survived the neurotoxic actions of CET are structurally altered. Several groups of investigators have used quantitative analysis of Golgi-impregnated hippocampal neurons to determine the effect of CET on measures of dendritic structure such as dendritic spine density and the extent and pattern of dendritic

branching. Predictably, the results have not always been entirely consistent because of differences across laboratories in several important variables. Some of these variables are as follows: (1) various species (mice, rats) and strains of animals are used that may differ in their response to the toxic actions of ethanol; (2) various methods and durations of ethanol exposure are used producing differences among studies in the length of ethanol exposure and the blood ethanol levels maintained; and (3) some investigators have used an ethanol-free recovery or withdrawal period following CET before data collection, and others have not. Nevertheless, during the past few years a clearer pattern of results has emerged regarding the effect of CET on the dendritic architecture of hippocampal neurons.

In an early study Riley and Walker (1978) used Golgi methods to examine the hippocampus of mice that received CET for 4 months, followed by 2 months of ethanol withdrawal. A substantial (50 to 60 percent) decrease was observed in the density of dendritic spines on basilar dendrites of CA1 pyramidal cells and proximal dendrites of DG granule cells. Subsequently it has been noted that CET reduces the spine size on dendrites of mouse hippocampal neurons (Lescaudron et al. 1989; Phillips and Cragg 1983). Phillips and Cragg (1983) found that many dendritic spines of the CET group were too small to be visible with the light microscope. It seems likely that we overestimated the extent of dendritic spine loss after CET in our early study (Riley and Walker 1978).

A careful analysis of the existing evidence suggests that hippocampal pyramidal cells and DG granule cells respond in an opposite manner to CET and subsequent ethanol withdrawal. During CET without a withdrawal period, the dendrites of surviving hippocampal CA1 pyramidal cells exhibit attenuated dendritic branching and/or reduced density of dendritic spines (King et al. 1988; Lescaudron et al. 1989; McMullen et al. 1984; Scheetz et al. 1987*b*). These regressive changes in dendritic structure progressively worsen as the duration of CET is increased but revert to near normal or recover completely during ethanol withdrawal (King et al. 1988; Lescaudron and Verna 1985; Lescaudron et al. 1989; McMullen et al. 1984). Conversely, the dendritic trees of granule cells in the DG that survive CET without a withdrawal or recovery period are found to show compensatory dendritic hypertrophy, that is, increased extent, increased branching, and increased spine density (Cadete-Leite et al. 1988*b*, 1989*a*; King et al. 1988). This dendritic hypertrophy regresses to age-matched control level during ethanol withdrawal (Durand et al. 1989; Cadete-Leite et al. 1989*a*; King et al. 1988). Both hippocampal pyramidal cells and dentate granule cells are killed during CET (vide supra). However, the surviving pyramidal cells respond with dendritic regression, perhaps due to deafferentation, whereas surviving granule cells may gain new connections, which are apparently lost after CET ceases. Validation of this hypothesis will require the use of electron microscopic autoradiography after increasing durations of CET and withdrawal.

Synaptic Density in Hippocampal Neuron Dendritic Fields After CET

There have been very few quantitative electron microscopic studies investigating the effect of CET on density of synapses onto hippocampal neurons. Lee et al. (1981) found no effect of 18 weeks of CET on the density of synapses innervating dendritic spines in the proximal stratum radiation. More recently it was reported that CET for 3 months reduced by 23 percent the density of spines and spine synapses in the stratum oriens of CA1 in LS but not in SS mice (Scheetz et al. 1987*b*). These results suggest again the possible role of genetics in the susceptibility to ethanol neurotoxicity. Cadete-Leite et al. (1989*b*) studied the effect of 6, 12, and 18 months of CET on the relative number of CA3 pyramidal cells and mossy fiber-CA3 synapses in the rat. CET produced a progressive loss of pyramidal cells and a significant decrease in mossy fiber-CA3 synapses. The CA3 cell loss was observed after 6 months or more of CET, but the number of mossy fiber-CA3 synapses did not decrease until 18 months. Since both dentate granule cells and CA3 cells are significantly reduced in number after 6 months of CET, the absence of synaptic loss after 6 to 12 months of CET suggests the formation of new synapses to compensate for the loss of projecting and target neurons.

CET and Reactive Synaptogenesis in the Hippocampus

The possibility that ethanol intake may limit morphological recovery from brain damage is suggested by reports that reversibility of cognitive performance and cerebral atrophy posttreatment (Carlen et al. 1984; Eckardt et al. 1980) significantly decreases after even moderate alcohol consumption in humans. It is generally recognized that neurons in the adult brain, the hippocampus in particular, can reorganize their synaptic connections in response to deafferentation resulting from removal of presynaptic elements by lesions or other perturbations. This process, called reactive synaptogenesis or axonal sprouting (Cotman and Nadler 1978), could underlie any morphological and functional recovery that can be achieved in chronic alcoholics. It is of interest whether ethanol exposure in controlled animal experiments alters the capacity for axonal sprouting in the hippocampus following partial deafferentation.

The experiments reported to date have examined the effect of ethanol on the extent of reactive synaptogenesis in the DG following partial deafferentation induced by a unilateral lesion of the entorhinal cortex (Lind et al. 1988; Orona et al. 1988; Tjossen et al. 1987; West et al. 1982). Removal of the entorhinal cortex eliminates most of the synapses in the outer two-thirds of the molecular layer of the ipsilateral DG (Cotman and Nadler 1978). This partial deafferentation normally results in the formation of new terminals of other intact afferent fibers.

Septohippocampal cholinergic terminals in the deafferented region are markedly increased, resulting in a marked intensification of acetylcholinesterase (AChE) staining. Cholinergic fibers also typically withdraw from the inner molecular

layer, resulting in decreased AChE staining or clearing in the proximal commissural/associational (C/A) zone, ipsilateral to the lesion. The width of the ipsilateral C/A zone is increased, reflecting an expansion of the territory of the C/A axons originating from contralateral and ipsilateral CA4 (Fricke and Cowan 1978).

The effect of ethanol on axonal reorganization, as measured by the extent of expansion of the C/A projection to the inner molecular layer, has been consistent among studies. Short-term ethanol exposure (10 to 40 days) administered following removal of the entorhinal afferents blocks or suppresses the extent of the typical lesion-induced expansion of the C/A zone (Lind et al. 1988; Orona et al. 1988; Tjossen et al. 1987; West et al. 1982). Interestingly, Lind et al. (1988) also found that abstinence from ethanol for only 24 hours released the suppression of lesion-induced C/A zone expansion, suggesting that the inhibition of axonal sprouting by ethanol requires the continued presence of ethanol.

Using this model, we have found that long-term ethanol exposure (20 weeks), given before unilateral removal of the entorhinal cortex, reduces the expansion of the C/A zone and reduces the condensation of AChE staining density in the outer molecular layer, ipsilateral to the lesion (Walker and Hunter 1987; King et al. personal communication). This finding suggests that prior long-term ethanol intake reduces both the sprouting of axons in CA4 afferents to the C/A zone and the sprouting of septohippocampal cholinergic afferents to the outer molecular layer. These results indicate that prior CET produces a residual alteration in the capacity for synaptic reorganization that lasts for at least 8 weeks after CET is discontinued. Given that CET for 12 weeks is reported to reduce the number of septohippocampal cholinergic neurons in the basal forebrain (Arendt et al. 1988*b*), it is possible that the capacity for cholinergic afferent sprouting could be limited.

Collectively the results described above support the conclusion that either a prior history of ethanol exposure or ethanol exposure after brain damage limits the capacity of surviving neurons to recover or reorganize. These results have important implications for the treatment of brain-damaged alcoholics as well as nonalcoholic brain-damaged patients.

SUMMARY AND CONCLUSIONS

The research summarized above provides definitive evidence that prolonged ethanol exposure results in altered structure and function of the hippocampus. The extent of neuronal loss ranges from 10 to 40 percent in various reports, depending on the duration of ethanol exposure, the magnitude and pattern of exposure (blood ethanol level maintained), the genetic susceptibility of the species and strain of animals studied, and the location within the hippocampus examined. The neurons surviving chronic ethanol exposure are also abnormal.

Quantitative analysis of dendritic structure in surviving neurons supports the conclusion that the principal cell types (pyramidal and granule cells) surviving CET respond differently to CET and its

withdrawal. The dendritic arborization and spine density of surviving pyramidal cells is attenuated during CET and recovers to normal during an ethanol-free withdrawal period. On the other hand, surviving granule cells show hypertrophy of dendritic extent and increased spine density during CET, which regresses to normal during ethanol abstinence.

We do not know if this dendritic regression of pyramidal cells during CET represents a response to deafferentation or dendritic dying back as a precursor to cell death. Nor do we know if the hypertrophy of dendritic structure observed in granule cells not lost during CET is a function of hyperinnervation due to axonal sprouting of afferents that have lost contacts with other cells. These questions are important for our understanding of the dynamic structural and functional changes occurring in the hippocampus during and after CET and should be pursued further.

Another important issue is why some cells within a class are lost during CET and others survive. Neurons in the hippocampus appear to be differentially susceptible to the toxic effects of ethanol both within and between cell types. Also, vulnerability of neurons of the same class appears to differ as a function of their location within the hippocampus. Further research on the mechanisms underlying this differential susceptibility of neurons to CET could provide important clues to help determine how ethanol exerts its neurotoxic actions.

There is a great interest in whether any recovery from ethanol-induced brain damage can be obtained by ethanol abstinence. Current information suggests that reorganizational and compensatory changes occur in the hippocampus during and following CET. However, ethanol exposure suppresses experimental deafferentation-induced axonal sprouting both before and after deafferenting lesions. The existence of reorganizational or compensatory changes in the hippocampus during and following CET does not necessarily support functional recovery. Indeed, both behavioral (vide supra) and physiological (Hunter, chapter 13) deficits are apparently irreversible. The importance of conducting parallel morphological and functional analysis of the effects of CET should therefore be emphasized. For example, even in the absence or presence of morphological recovery or compensation, surviving afferent connections could exhibit changes in synaptic function that would not be detected morphologically (Hunter, chapter 13).

Substantial progress during the last few years has been achieved in expanding our knowledge of the effects of prolonged ethanol exposure on the structure of the hippocampus. We still do not know how these structural changes relate to the functional disturbances of memory produced by CET, nor do we understand the mechanisms of ethanol neurotoxicity. Mechanistic hypotheses are now being tested (see Mechanisms and Therapeutic Strategies Section). Hopefully, these studies will increase our knowledge of how ethanol exerts its neurotoxic action.

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12

ALTERATIONS IN THE STRUCTURE OF THE CEREBELLUM AFTER LONG-TERM ETHANOL CONSUMPTION

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INTRODUCTION

Long-term alcohol abuse is known to have effects on motor coordination and equilibrium, resulting in motor disorders of variable severity. In human abusers, the more readily recognized behavioral expressions of the toxic effects of chronic alcohol abuse on the central nervous system (CNS) are a broad-based gait and a loss of power in muscular coordination (ataxia), causing lurching and staggering. These movement disorders are usually associated with cerebellar damage (Neiman et al. 1990). Ethanol-related atrophy of neurons in midline portions of the cerebellum was well-documented years ago (Victor et al. 1959), but the means by which ethanol causes neuronal degeneration in the cerebellum and the mechanisms that link cell degeneration to specific movement disorders are not known. In one recent postmortem study, alcoholic cerebellar degeneration was found in 42 percent of male alcoholics studied (Torvik and Torp 1986), but subclinical expressions of this disease may be even higher.

Obtaining sufficient information to remove the gaps in our understanding of the neurotoxic effects of ethanol on the cerebellum is extremely difficult from studying human patients alone. Consequently, investigators have turned to well-controlled studies of chronic ethanol consumption in animal models. In this chapter, the contributions from studies with rodent models will be presented. During the past 15 years, we have learned a great deal from such studies, but it will also become apparent that a great deal more study will be required to understand fully how ethanol alters neuronal structure(s) in the cerebellum and the functional consequences of these alterations. This chapter is in effect a progress report, updated but still incomplete.

THE RODENT MODEL AND ETHANOL STUDIES

Most investigators use rodents to study ethanol-associated structural changes in neurons because of excellent methods developed for administering ethanol to rodents over long periods in physiologically

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relevant amounts. All the information presented in this chapter has been derived from experiments in rodent models.

McClearn (1988) has pointed out that the use of animals to study a pathological process occurring in humans involves two important questions that must be answered. First, is the experimental paradigm adequate to measure that pathological process in the animal, and second, do the results obtained from study of that animal supply information that relates directly to the same pathological process in humans?

In answer to the first question, it has been demonstrated repeatedly that when dietary ethanol is administered continuously to rodents for sufficiently long periods, permanent structural alterations are produced in brain tissues. This is particularly true for the rodent cerebellum, which appears to be one of the most ethanol-sensitive regions of the rodent brain (Walker et al. 1981). The answer to the first question then is that cerebellar pathology similar to that seen in humans can be induced in rodents.

The pattern of cerebellar degeneration induced by carefully controlled ethanol administration in rodent models differs in some respects from that described in autopsied tissue from human ethanol abusers (Charness et al. 1989). When all the available data are reviewed in a comprehensive way, however, as we shall do here, those differences may be seen as more apparent than real. Some pathologies associated with long-term human abuse of ethanol may be less severe in rodents simply because a period longer than the life-span of the rodent is required for their full expression.

In answer to the second question, ethanol-induced cerebellar damage in the rodent model is accompanied by behavioral correlates similar to the movement disorders associated with human cerebellar degeneration. We should not expect such disorders in rodents to be identical to those in humans due to obvious differences between bipedal and quadrupedal locomotion. However, there should be some correspondence between the behavioral changes in the model and those in humans.

A form of ethanol-associated ataxia does occur in the rodent model. The behavior that is affected is the righting reflex, the natural tendency of an animal to right itself on four feet when placed on its back. The duration between the loss and return of that reflex following a single injection of ethanol is called sleep-time. It has been shown that sleep-time has a high genetic correlation with the sensitivity of cerebellar Purkinje neurons to the depressant effects of ethanol (Palmer et al. 1987). The greater the sensitivity of the Purkinje neuron, the longer will be the sleep-time. Permanent modifications of gait, corresponding to the broad-based gait associated with chronic ethanol abuse in humans, have been found in rats after disruptions in cerebellar development induced shortly after birth by ethanol (Meyer et al. 1990). However, motor abnormalities after long-term ethanol exposure in adult rodents have not yet been reported. It appears then that the rodent does, in fact, develop appropriate behavioral correlates for study

of the effects of ethanol on cerebellar structure/function.

It must be acknowledged that the pattern of ethanol administration to rodents does not perfectly correspond with the patterns of ethanol consumption by human alcohol abusers. A scheduled daily intake of ethanol in approximately constant amounts does not mimic very closely the variable patterns of ethanol consumption by human abusers. In addition, whereas clinically relevant blood ethanol levels of 100 to 150 mg/dL are commonly produced in ethanol-consuming rodents on a nutritionally adequate liquid diet (Lieber et al. 1989), higher blood levels (200 to 300 mg/dL) are produced in humans during 45 consecutive days of chronic intoxication under nutritionally controlled conditions (Isbell et al. 1955). But, when human blood ethanol levels averaged 200 to 300 mg/dL, signs of withdrawal also appeared (Mello and Mendelson 1971), a complication that can be avoided in controlled experiments with rodents.

Appropriate controls are an essential part of a well-designed model. If the ethanol is administered in the drinking water (usually a 15- to 20-percent concentration of ethanol), a control for nutrition and weight gain must be used. If the ethanol is administered in a nutritionally adequate liquid diet (35 to 40 percent of the calories supplied by ethanol), two controls should be used, an isocaloric control for effects of the ethanol and a standard (chow) control for the effects of the liquid diet per se. Throughout the discussion of ethanol-associated structural changes below, all of the structures altered during ethanol treatment differ significantly from corresponding structures in neurons of the appropriate control rats (for further discussion of experimental design, see Walker et al., chapter 11).

STRUCTURAL FEATURES OF THE CEREBELLUM

The cerebellum is a prominent cortical structure that lies above the brain stem within the posterior concavity of the skull. It is a single structure, physically separated from the paired cerebral hemispheres. In addition, it lies almost entirely free within the posterior portion of the skull, anchored to the underlying brain stem by three large pairs of nerve fiber bundles, called cerebellar peduncles. Viewed externally (figure 1A), the cerebellum is seen as distinctly lobed. A central midline lobe, the vermis, is flanked by two prominent lateral lobes, the cerebellar hemispheres. In rodents, a surface depression marks the lateral boundaries of the vermis, separating it clearly from the adjacent hemispheres. The cerebellum also has a series of prominent folds that run laterally across the cerebellar surface and that divide each lobe into smaller subunits called lobules (figure 1B).

The basic organization of the cerebellum is similar in all mammals, including humans, with species differences being reflected largely in the overall size of the cerebellar lobes and in the complexity of folding in the cerebellar lobules. Neurons and their supporting glial cells make up the cell populations intrinsic to the cere-

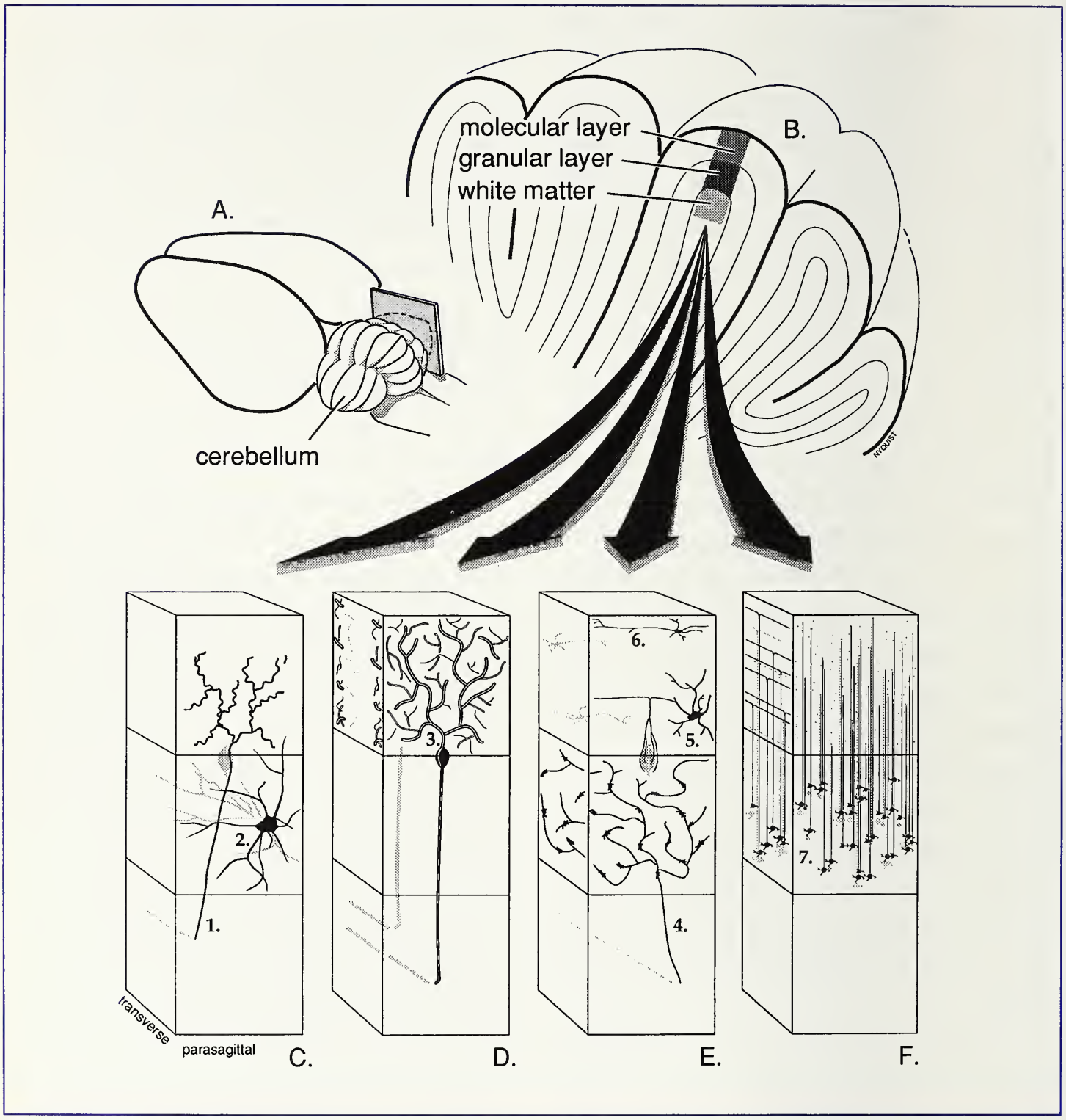

FIGURE 1

A, External view of the cerebellum. B, Schematic drawing showing the parasagittal view of the layering and lobular subdivisions of the cerebellar cortex. The parasagittal surface was exposed by sectioning along the shaded plane in A. C-F, Repeated views of the same shaded block of tissue in B, illustrating separately the major afferent fibers and the major types of cerebellar cortical neurons: 1, climbing fiber (Purkinje neuron cell body is shaded in for orientation); 2, Golgi neuron; 3, Purkinje neuron; 4, mossy fiber; 5, basket neuron (Purkinje neuron cell body is shaded in for orientation); 6, stellate neuron; and 7, granule neurons with axons extending into the molecular layer as parallel fibers.

bellum. However, neurons are distinguished from glial cells both structurally and functionally. Neurons have well-developed dendrites and axons that extend out from their cell body and are specialized structurally for unidirectional

transmission of information within the nervous system. Dendrites provide approximately 95 percent of the receptive surface of a neuron and, when adequately stimulated, transmit information to the cell body of the neuron for processing. An axon transmits information away from the neuronal cell body to a second cell. The second cell may be either excited or inhibited, an effect usually achieved through axonal release of a neurotransmitter that is recognized by receptor molecules on the dendritic membrane of the second cell.

The types of neurons that populate the cerebellar cortex are similar in all mammalian species with species differences being reflected mainly in the complexity of the branching processes of the neurons. The uniformity of cerebellar organization, the relative simplicity of its organization compared with that in other types of cortex, and the versatility of its structure based upon a modular organization (discussed below) have made this subdivision of the brain the region of choice for numerous studies of neuronal structure and function. The frequent association between alcohol abuse and cerebellar atrophy adds to the rationale for its use to study the effects of ethanol on neurons.

In all types of cortex, the intrinsic neurons, dendrites, and axons are organized into layers. The layered organization of the cerebellar cortex is appreciated best when viewed in the parasagittal plane (a plane parallel to a midline cut that separates the brain into right and left halves), as illustrated in figure 1B. The cerebellar cortex has only three layers, making it the simplest form of cortex in the mammalian CNS. These layers are easily distinguished from one another. The superficial molecular layer is pale in appearance, because it contains few, widely spaced neurons surrounded by immense numbers of axonal and dendritic processes.

In contrast, the layer immediately below the molecular layer, the granular layer, contains vast numbers of closely packed, small neurons (granule neurons). The distinct border between the fibrous molecular layer and the cellular granular layer is occupied by a single layer of very large neurons called Purkinje neurons that have prominent oval cell bodies with elaborately branched dendrites (figure 1D). Below the granular layer is a third layer called the white matter. The white matter contains bundles of myelinated axons that enter and exit the cerebellum.

Axons entering the cerebellar cortex are called afferent fibers, and those exiting the cerebellar cortex are called efferent fibers. Three categories of afferent axons—climbing fibers, mossy fibers, and monoaminergic fibers—enter the cerebellar cortex, but only one category of efferent axons—Purkinje cell axons—exits the cortex.

Climbing Fibers

Climbing fibers arise from neurons in the inferior olivary nucleus in the brain stem. Each climbing fiber passes through the granular layer of the cerebellar cortex to establish a one-to-one relationship with a single Purkinje neuron. The climbing fiber mimics faithfully the branching pattern of the main dendrites of the Purkinje

neuron, forming multiple synaptic contacts with those dendrites. However, it does not extend along the small-diameter peripheral branches of the Purkinje neuron (figure 1C). The climbing fiber is the only direct route to the Purkinje neuron from brain stem centers, and through its multiple synaptic contacts, it provides powerful excitatory stimulation to that neuron.

Mossy Fibers

Mossy fibers are so named because of the moss-like terminals that they form in the granular layer. These fibers arise from several sources outside the cerebellar cortex, which will be referred to here simply as precerebellar nuclei. Mossy fibers are very numerous, accounting for about two-thirds of the nerve fibers in the white matter. They extend only into the granular layer, where each mossy fiber branches to form complicated swollen terminals (rosettes), each of which synapses with dendrites of many granule neurons (figure 1E). The excitatory activity of the mossy fibers is then relayed indirectly to the Purkinje neurons by the granule neurons.

Monoaminergic Fibers

Monoaminergic afferents (noradrenergic, serotonergic, and less well-defined dopaminergic afferents) from brain stem reticular nuclei (the locus coeruleus and the raphe complex) are distributed diffusely to all parts of the cerebellar cortex. These cerebellar afferents will not be considered further here, because no data are available concerning ethanol-induced structural changes in these axons in the cerebellum.

Purkinje Cell Axons

Purkinje cell axons provide the only efferent path from the cerebellar cortex. Most of these axons transmit the processed information from the cortex to four groups of neurons (deep cerebellar nuclei) embedded in the white matter directly below the cerebellar cortex on each side of the midline. The deep cerebellar nuclei subsequently relay that information to brain stem centers for a precise distribution to the spinal cord and cerebral cortex.

Intrinsic Cerebellar Circuitry

The structural organization of the neurons in the cerebellar cortex—its "machinery" connected to the input (afferent) and output (efferent) pathways—is the same throughout all the lobules and lobes. Consequently, the intrinsic circuitry of the cerebellar cortex can be considered as composed of repeating modular units, analogous to computer chips. The fact that this intrinsic circuitry is similar in all mammals underlies our expectation that information from a rodent model may relate immediately to the functioning of human cerebellar cortex. Modular units with similar function, i.e., with similar afferent and efferent connections, are clustered in what are termed "microzones." Our ability to define the specific functions of different areas in the cerebellar cortex is based on our ability to identify microzones. Functional differences assigned to specific lobules and lobes do not stem from differences in the organization of their intrinsic neurons, but from differences in the input and output connections of their microzones.

In humans, the cerebellar cortex is much more expansive than in the rat because phylogenetically new parts of the cerebellum have enlarged in parallel with expansion of the association cortex in the cerebral hemispheres. There are massive reciprocal connections through relay centers in the brain stem between the expanded association cortex and this new (neo) cerebellar cortex. The appearance of these "new" anatomical connections in the human brain means that the functional repertoire of the cerebellum can no longer be considered related only to motor skills. There is mounting evidence that the lateral cerebellum may be involved in human cognitive and language functions (Decety et al. 1990; Leiner et al. 1991). Recent studies have also suggested that even in animals, the cerebellum may be involved in spatial learning and discrimination learning (Lalonde and Botez 1990).

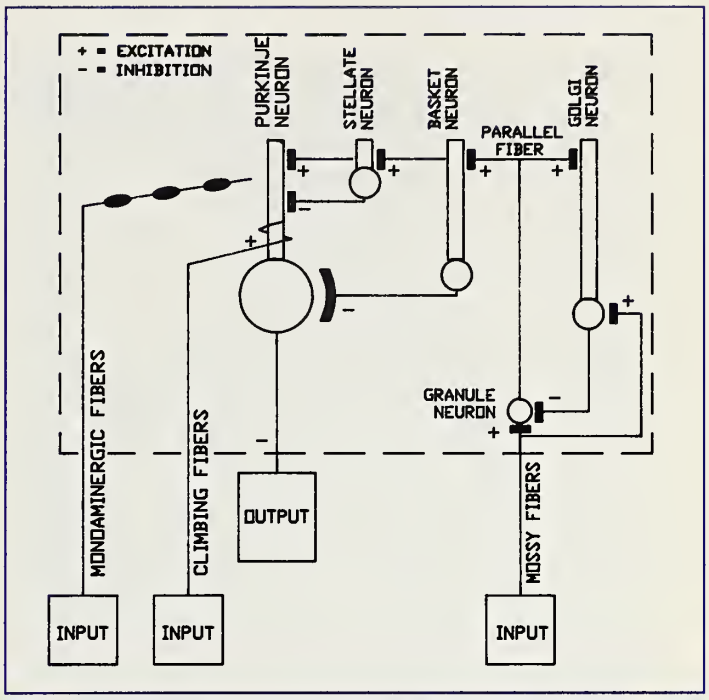

FIGURE 2

Diagram illustrating the modular organization of cerebellar neurons. Purkinje neurons receive direct excitatory input from climbing fibers and indirect excitatory input from mossy fibers by way of granule neurons and their parallel fibers. Stellate neurons and basket neurons participate in inhibitory loops that feed back to Purkinje dendrites and to the Purkinje cell body and initial segment of the axon, respectively. Golgi neurons participate in an inhibitory loop that feeds back to granule cell dendrites. The Purkinje cell axon provides the only efferent pathway from the cerebellar cortex. (Collateral branches of monoaminergic fibers, climbing fibers, mossy fibers, and Purkinje cell axons have been omitted for clarity.)

Modular Organization

The intrinsic modular units of the cerebellar cortex are constructed from five types of neurons situated entirely within the cerebellar cortex (figure 2). The Purkinje neuron occupies a key position as the only exiting path from each module (figure 2). Purkinje neurons have an elaborate tree-like dendritic network that extends throughout the depth of the molecular layer. In the rodent, the dendritic network of a single Purkinje neuron may have as many as 1,600 to 1,700 branching segments, making it one of the largest and most complex neurons encountered anywhere in the CNS (figure 3A). Each dendrite also forms many thorn-like protrusions (spines) (figure 3B), which serve as specialized sites of synaptic contact with axons of other neurons. A peculiar fact about the Purkinje neuron is that its dendritic network fans out in the molecular layer in a relatively thin plane. As a result, the cell's lateral boundaries may be 300 to 350 µm apart, whereas its depth of branching may be only 30 to 40 µm. When the cerebellar cortex is sectioned in the parasagittal plane, the full lateral extent of each Purkinje network can be seen (figure 1D). When the cerebellar

FIGURE 3

A, Purkinje neuron in an 11-month-old control rat, lobule VII. Note extensive dendritic branching near the surface of the molecular layer (arrowheads). x245. B, Golgi-Kopsch impregnation of Purkinje cell dendrites showing spines on terminal and next-to-terminal segments. x2,500. Reproduced with permission from Pentney (1986).

cortex is sectioned in a transverse plane (perpendicular to the sagittal plane), the limited depth of the Purkinje cell network is viewed.

The granule neuron has a small cell body, approximately the size of a red blood cell, making it one of the smallest neurons found in the CNS. What it lacks in size, however, it makes up for in numbers. It has been estimated that cerebellar granule neurons account for seven-eighths of all neurons in the CNS (Williams and Herrup 1988). Each granule neuron forms two to seven short dendritic processes and a single long axonal process that extends up into the molecular layer.

Within the molecular layer, each axon bifurcates to produce two processes that extend parallel to the surface of the molecular layer and perpendicular to the expansive Purkinje dendritic networks (figure 1F). Because these axons are so precisely oriented, they are called parallel fibers. Each T-shaped parallel fiber intersects a series of Purkinje cell networks as it courses through the molecular layer in both directions from its point of bifurcation. As parallel fibers pass through the Purkinje dendritic networks, they form synapses with spines on the small-diameter, Purkinje cell dendrites. The parallel fibers are a second major source of excitatory stimulation to the Purkinje neurons (figure 2).

Recognition of other types of interneurons—stellate cells, basket cells, and Golgi cells—is assisted by the segregation of these cells into one of two layers. The cell bodies of stellate cells and basket cells reside within the molecular layer (figure 1E), and those of Golgi neurons, within the granular layer (figure 1C). The stellate cells and basket cells are further segregated within the molecular layer so that stellate cells occupy the upper two-thirds and the basket cells are restricted to the lower one-third of the molecular layer. Parallel fibers supply 100 and 92 percent of the excitatory input to stellate cell dendrites and cell bodies, respectively. The percentages for parallel fiber excitatory input to basket neurons are slightly lower, 89 and 74 percent, respectively (Lemkey-Johnston and Larramendi 1968*a*,*b*).

Golgi neurons in the granular layer have a more varied type of input. Golgi neurons are excited by mossy fibers, parallel fibers, and climbing fibers (not shown in figure 2), whereas they are inhibited by recurrent collaterals of Purkinje cell axons, stellate cell axons, and basket cell axons (none of these is included in figure 2). These three interneurons have an inhibitory effect on their target neurons: the stellate neurons, on Purkinje cell dendrites; the basket neurons, on the Purkinje cell body and initial segment of its axon; and the Golgi neurons, on granule cell dendrites (figure 2).

The relatively complete segregation of neurons into specific layers, as described above, makes neuronal identification simpler in cerebellar cortex than in other types of cortex. This is an especially helpful feature when neurons are structurally modified by pathological processes, making them more difficult to identify. It is safe to say that the cerebellar cortex has been studied to a greater extent than any other type of cortex, precisely because it has such a simple, layered arrangement with a limited number of different types of neurons, distributed in easily recognized patterns within the layers. As a result, the relations between cytoarchitecture and physiology are better understood for the cerebellar cortex than for other types of cortex.

Several models have been developed to explain the significance of the highly ordered modular arrangement of cerebellar neurons. The bulk of current experimental evidence supports a model that combines spatial pattern discrimination [proposed by Marr (1969) and Albus (1971)] with temporal pattern discrimination [adaptive filter model proposed by Fujita (1982)].[2]

ETHANOL-INDUCED DECREASES IN NEURONAL NUMBERS

Most studies of the effects of chronic ethanol treatment on cerebellar neurons have focused on the neurons that are integral to the structural module described above. Two reports from one laboratory showed that different types of neurons had different degrees of sensitivity to chronic ethanol consumption. Following chronic administration of aqueous

[2] *For further discussion of these models the reader is referred to Ito (1984).*

ethanol, granule cell number and stellate cell number were significantly reduced after 6 months of ethanol treatment (Tavares and Paula-Barbosa 1982). On the other hand, significant decreases in basket cell number and Purkinje cell number required 12 and 18 months of treatment, respectively. Golgi cell number was stable even after 18 months of treatment (Tavares et al. 1987*a*). When a nutritionally complete liquid diet was used as the vehicle for chronic ethanol administration, however, Purkinje neurons as well as granule neurons were reduced in number after 2 months of recovery following 5 months of ethanol treatment (Walker et al. 1980). In this study, the total number of Purkinje cells in a midline section of the cerebellum was significantly reduced by 20 to 25 percent. Significant decreases of 20 to 25 percent were also measured in the granule cell population in that section and in the area of the molecular layer, reflecting a loss in Purkinje cell dendrites and in granule cell axons.

The results reported by Tavares et al. (1987*a*) did not exactly duplicate the changes described in humans with cerebellar degeneration. The predominant abnormality in humans was a severe decline in the numbers of Purkinje neurons (Victor et al. 1959). The severity of changes in the human Purkinje neuron population following chronic abuse of ethanol may, however, reflect the longer duration of abuse possible during the human lifespan, rather than an essential difference in human Purkinje cell sensitivity to ethanol. Results from the study of Walker et al. (1980), on the other hand, were more consistent with descriptions of human alcoholic cerebellar degeneration.

There may, of course, be a real difference in the sensitivity of these neurons in different strains of rats, but several methodological differences in these two studies could also be sources of the difference. The volume of ethanol consumed by a rat when the ethanol is part of a liquid diet providing its sole source of calories is generally larger than that consumed when ethanol is given in the drinking water. Circulating ethanol levels, therefore, were probably different in the two studies. Blood ethanol levels were not measured by Tavares et al. (1987*a*), but these investigators reported that their rats consumed approximately 9 ± 1.3 g ethanol/kg body weight/day (Tavares and Paula-Barbosa 1982), whereas Walker et al. (1980) reported an average daily intake of approximately 12 to 15 g/kg/day by their rats. High blood ethanol levels in the rats used by Walker et al. (1980) may explain the earlier occurrence of significant Purkinje cell loss in their study. The lower ethanol intakes may have been effective in discriminating between different thresholds for neuronal sensitivity to ethanol, whereas the higher intakes may have exceeded all thresholds.

There were also differences in the procedures used to quantitate the neuronal populations. Tavares et al. (1987*a*) measured the total number of each neuronal type within the entire volume of the cerebellum, whereas Walker et al. (1980) estimated cell numbers from a single midline section in each cerebellum. Since the total number of granule neurons and

Purkinje neurons in the cerebellum was not determined in the latter study, the possibility that distributional changes occurred within the Purkinje cell layer, giving the appearance of cell loss without actual loss of cells, cannot be excluded.

The use of a recovery period following the ethanol treatment also needs to be evaluated. In theory, an ethanol-induced cell loss should be unaffected by a subsequent recovery period. However, Phillips and Cragg (1984) reported that a significant loss of mouse cerebellar Purkinje neurons did occur during a 4-month recovery period that followed 4 months of ethanol treatment. Their data suggested that the withdrawal process itself contributed to the death of Purkinje neurons and introduced the possibility that some of the Purkinje cell loss reported by Walker et al. (1980) may have occurred during the recovery period.

Documenting a loss of neurons in a cell population is easier than identifying the underlying mechanisms. If we hypothesize that intrinsic neuronal organization or metabolism influences neuronal sensitivity to ethanol, ethanol-associated neuronal regression should occur in each cell population independently. If, however, we hypothesize that neuronal loss occurred initially in the granule cell population of the cerebellar cortex, as the results reported by Tavares et al. (1987*a*) suggest, and that regression of all other types of cerebellar neurons occurred transsynaptically because of parallel fiber regression, we might expect that the rapidity and extent of regression in deafferented neurons would reflect their degree of dependence upon parallel fiber innervation. When ranked according to degree of dependence upon parallel fiber innervation, stellate neurons would be ranked first, basket neurons second, and Purkinje neurons third, the sequence of cell loss reported by Tavares et al. (1987*a*). These considerations lead us to examine what further evidence is available to support these two hypotheses.

ETHANOL-INDUCED ULTRASTRUCTURAL CHANGES: INCLUSIONS/ORGANELLES

Several ethanol-associated changes in ultrastructural characteristics of cerebellar cortical neurons have been identified in electron micrographs. They include unusual intranuclear and dendritic inclusions, degenerative changes in mitochondria, and premature accumulations of lipofuscin pigment in the neurons.

Intranuclear Inclusions

Intranuclear inclusions appeared as rods or sheets within the nucleoplasm of granule neurons, stellate neurons, and Purkinje neurons of rats treated with ethanol for 6 months (Tavares and Paula-Barbosa 1981). The frequency of occurrence was 1 per 1,000 granule cell nuclei, 27 per 1,000 Purkinje cell nuclei, and 3 per 100 stellate cells. None was found in Golgi neurons or in neurons of rats treated for only 3 months. Intranuclear inclusions similar to those reported by Tavares and Paula-Barbosa (1981) were described by Peters et al. (1970) as composed of filaments, 50 to 70 x 10^{-1} nm in diameter, grouped in rod-like structures or

arranged in parallel sets to form a sheet resembling a crystalline lattice. These structures are uncommon, but they have been seen in a variety of normal neurons, including cerebellar neurons (Siegesmund et al. 1964), suggesting that they occur widely although infrequently in all neurons (Peters et al. 1970). It is unlikely, therefore, that they represent a direct effect of ethanol treatment. Their presence in neurons of ethanol-treated rats suggests that they are secondary and indirect consequences of an altered neuronal metabolism.

Dendritic Inclusions

Dendritic inclusions appeared as paired, apposed membranes arranged in unusual whorled and multiplanar shapes (Tavares et al. 1985*c*). These novel structures were observed only in Golgi neurons, suggesting that Golgi neurons were not entirely unaffected by circulating ethanol. In approximately 1 percent of Golgi cell dendrites, these unique inclusions appeared continuous with smooth endoplasmic reticulum (SER) in the dendrite. It is well known that one of the functions of the liver cell SER is detoxification of drugs. Liver cell SER will proliferate when exposed to high levels of circulating ethanol. There is no evidence currently that the SER of Golgi neurons functions like that in liver cells, but it is tempting to speculate that the greater resistance of the Golgi neuron to ethanol (Tavares et al. 1987*a*) may be a consequence of a more responsive or more efficient SER in this cell type.

Structural Abnormalities in Mitochondria

Mitochondria provide the chemical energy required for the biosynthetic and motor activities of the neuron. Mitochondria are bounded by two membranes. The smooth outer membrane isolates the interior of the organelle from the surrounding neuronal cytoplasm. The inner membrane is highly folded to produce membranous shelves containing repeating assemblies of multienzyme systems that drive energy-producing reactions.

Extensive mitochondrial damage was detected in cell bodies and dendrites of rat Purkinje neurons after 10 weeks of treatment with a liquid ethanol diet (Pentney 1979). Dissolution of the mitochondrial cristae to varying degrees was the usual marker of mitochondrial degeneration, but there was also evidence of swelling with bizarre deviations from normal mitochondrial structure (figure 4). Tavares and Paula-Barbosa (1983*a*) reported as well that aqueous ethanol treatment for 3 and 6 months was associated with an increase in the volume density of the mitochondrial profiles in Purkinje neurons. This enlargement did not seem a result of swelling because there were no structural breaks in the mitochondrial membranes or the cristae. After 12 and 18 months of ethanol intake, however, misshapen mitochondria were observed (figure 5). As discussed above on differences in the timing of Purkinje cell loss after intake of aqueous ethanol versus a liquid ethanol diet, an advanced stage of mitochondrial damage occurred earlier when a liquid ethanol diet was used (Pentney 1979).

Mitochondrial damage in liver cells is a well-known consequence of alcohol abuse (Kiessling and Tobe 1964; Porta et al. 1965). Since the liver is the primary site of ethanol metabolism in the body, there is a direct relationship between altered liver cell structure and function. French (1968) found that, after chronic aqueous ethanol intake, the structural integrity of mitochondria in rat liver cells was compromised by loss of the outer membranes and cristae, resulting in increased mitochondrial fragility. Neuronal mitochondria are not involved directly in ethanol metabolism, however, and the metabolic consequences of ethanol-induced changes in their structure remain to be shown.

Advanced Formation of Lipofuscin Granules

A second type of organelle, the lipofuscin granule, has been proposed as a marker for ethanol-induced modifications of metabolism in Purkinje neurons (Tavares and Paula-Barbosa 1983*b*), granule neurons, Golgi neurons, and basket neurons in the cerebellar cortex (Tavares et al.

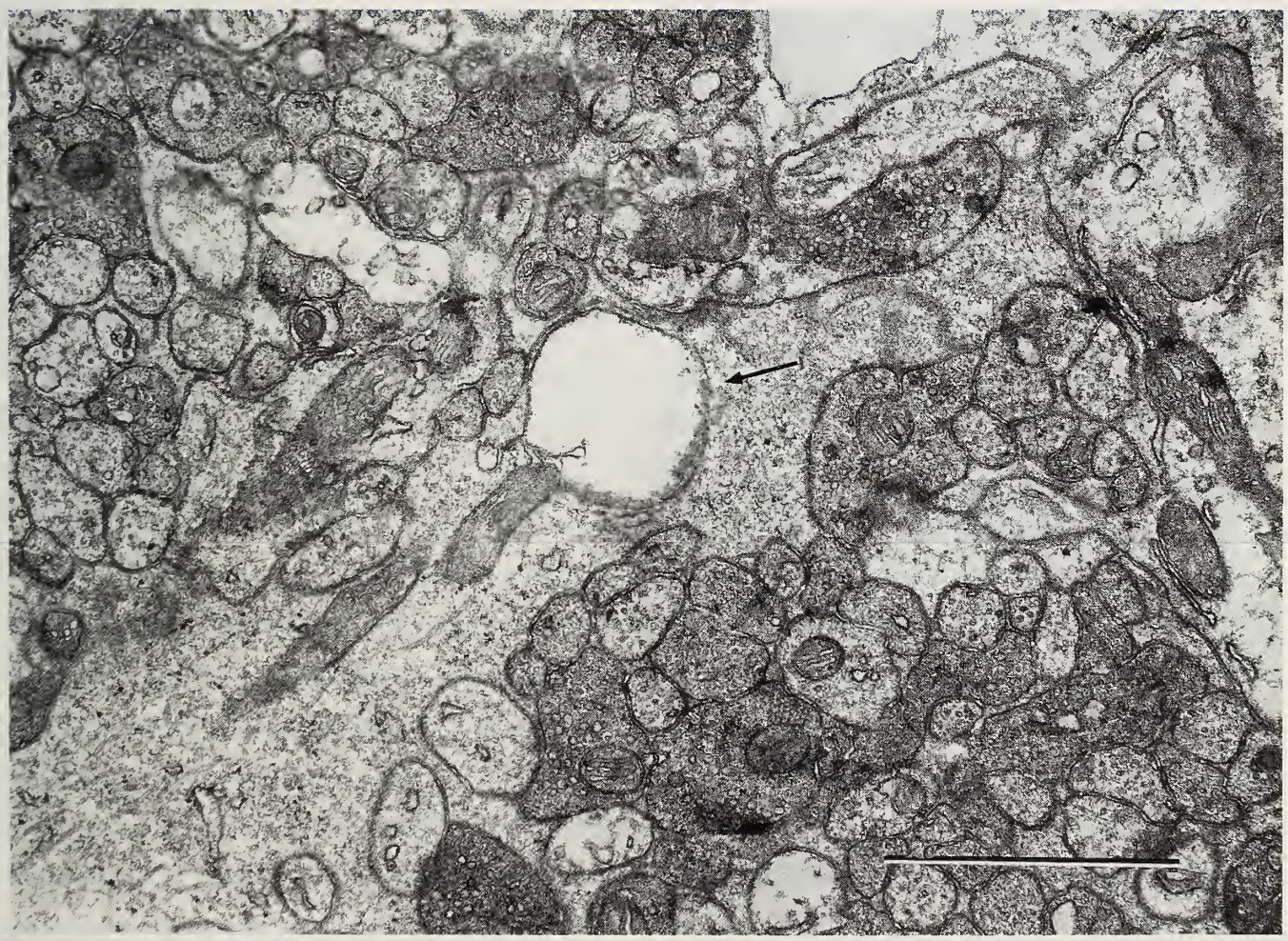

FIGURE 4

A degenerating mitochondrion (arrow) in a Purkinje cell dendrite of a 14-month-old rat fed an ethanol diet for 10 weeks. Calibration bar: 0.5 μm.

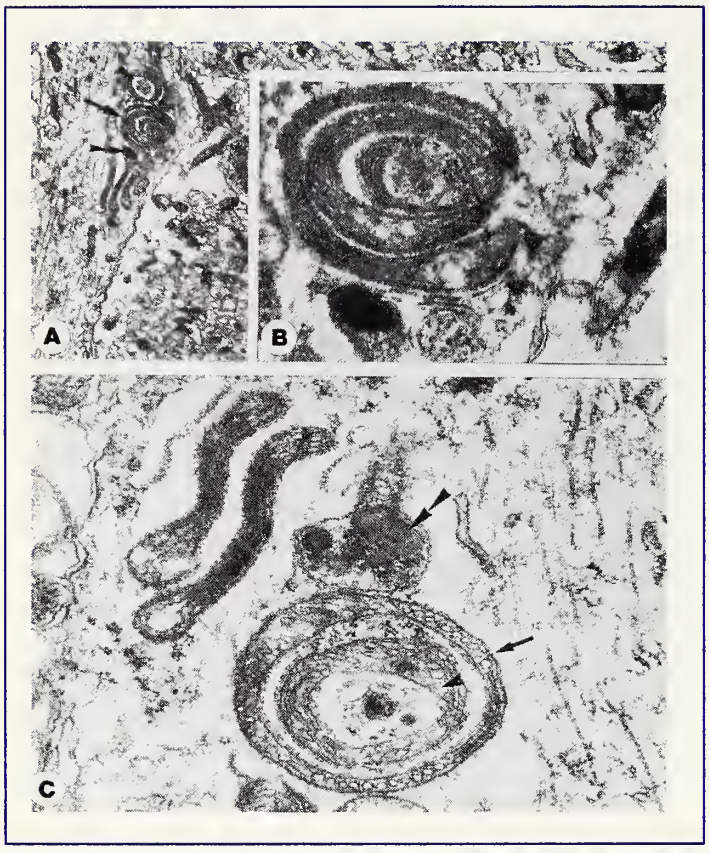

FIGURE 5

A, Proximal part of the apical dendrite of a Purkinje cell from a rat fed aqueous ethanol for 12 months. Groups of ring-shaped mitochondria wrapping others (arrow) and engulfing portions of cytoplasm (arrowhead) are observed. An electron-dense deposit in one mitochondrion is also noted (double arrowhead). x7,200. B, Higher magnification of the mitochondrial profiles in A. x48,000. C, A large mitochondrion (arrow) from a rat given aqueous ethanol for 18 months. The mitochondrion is wrapped around another mitochondrion (single arrowhead) that engulfs a portion of cytoplasm. A nearby mitochondrion (double arrowheads) has a matrix filled by electron-dense deposits (double arrowheads). x48,000. Reproduced with permission from Tavares and Paula-Barbosa (1983*a*).

1985*a*). Pigmented lipofuscin granules have been associated with cellular aging for many years and are frequently termed "aging pigment." The presence of lipofuscin granules in neurons is not unusual. Although those that accumulate in neurons of ethanol-treated rats do not show any unusual changes in structure, their early appearance and their increased numbers in neurons of young ethanol-treated rats suggests a possible link of these organelles to the ethanol treatment.

It was suggested, further, that since lipofuscin accumulates during normal aging and during chronic ethanol treatment, long-term ethanol consumption may be closely related to precocious neuronal aging (Tavares et al. 1985*a*). A direct causal connection between lipofuscin deposition and neuronal regression has not, however, been established for normal aging (Brody and Vijayashankar 1977). In addition, several other environmental factors, such as chronic vitamin E deficiency or chronic hypoxia, also lead to precocious and excessive deposition of lipofuscin pigment in neurons of young rats (Sulkin 1958). For these reasons, evidence is presently insufficient to support that suggestion.

Lipofuscin pigment granules appear to be the end products of enzymatic activities within lysosomes (Brunk and Ericsson 1972). The critical enzymatic reaction in the formation of lipofuscin is polyunsaturated lipid peroxidation (Tappel 1970). The demonstration that piracetam could decrease the amount of ethanol-associated lipofuscin deposited in Purkinje neurons (Paula-Barbosa et al. 1991) was not surprising, therefore, given the anti-oxidizing properties of piracetam.

The ethanol-associated ultrastructural modifications presented above may have contributed to cell loss in different populations of cerebellar neurons. The mitochondrial abnormalities were considered most likely to lead to subsequent regression of other neuronal structures

and eventual cell death. None of the unusual or altered structures could, however, explain the variability in neuronal sensitivity to ethanol shown in the studies reported by Tavares et al. (1987*a*). Mitochondrial abnormalities were described only in more resistant Purkinje neurons. Mitochondrial structure in other types of cerebellar neurons has not been studied in relation to ethanol treatment. As for the intranuclear and dendritic inclusions and the lipofuscin granules, they were either too widespread in occurrence (intranuclear inclusions and lipofuscin granules) or were seen only in the relatively ethanol-insensitive Golgi neuron (dendritic inclusions).

ETHANOL-INDUCED CHANGES IN NEURONAL PROCESSES

Regression of Granule Cell Dendrites

The fact that granule neurons decreased in number after 6 months of ethanol treatment (Tavares and Paula-Barbosa 1982) suggested that degenerating dendrites of granule neurons should be present in the granular layer during the same period. To document regression of granule cell dendrites, Tavares and Paula-Barbosa (1984) examined electron micrographs of cerebellar glomeruli in the granular layer of ethanol-treated rats.

Cerebellar glomeruli are structural units composed of mossy fiber rosettes, surrounded by granule cell dendrites and Golgi cell axon terminals. The mossy fiber exerts an excitatory action on the granule cell dendrites (and on nearby cell bodies of Golgi neurons), and the axon terminals of the Golgi neurons exert an inhibitory action on the granule cell dendrites. In the glomerulus, therefore, the mossy fiber and the Golgi cell axon terminals are presynaptic structures, and the granule cell dendrites are postsynaptic to them.

Tavares and Paula-Barbosa (1984) reported that the area of the glomeruli and the number of synapses in the glomeruli were unchanged after ethanol treatment, but a random patchy distribution of degenerating granule cell dendrites was identified. The dendritic degeneration coincided exactly in time of appearance with the previously reported decline in granule neurons (Tavares and Paula-Barbosa 1982). The degenerating dendrites were not in continuity with degenerating cell bodies, but they were in close proximity. After 12 to 18 months of ethanol treatment, degenerating granule cell dendrites were no longer prominent in the tissue, and granule cell dendrites were almost entirely absent from some glomeruli. In other glomeruli, regressed granule cell dendrites were apparently replaced by dendrites of Golgi neurons, an unusual synaptic association in the glomeruli (Tavares and Paula-Barbosa 1984). Possible consequences of Golgi neuron hypertrophy will be discussed in a later section.

Regression of Neuronal Dendrites

Several studies have suggested that lengthy ethanol consumption may affect dendritic branching patterns, lengths, and spine densities, changes that certainly modify the functional capabilities of neurons. Studies of some of these dendritic

properties require that entire dendritic networks be visualized in the tissue sections. This is easily accomplished in Golgi preparations.

In all modifications of the Golgi method, neural tissue is exposed to either silver or mercury salts under conditions that will promote precipitation of metallic complexes within a small percentage of the neuronal population. It is not unusual for the precipitate to fill all parts of a neuron completely. However, why only a small percentage of the neurons are usually stained by these procedures is not known, but this fact is what makes the method useful. It is only because most of the neurons are unstained that we can study the stained ones. Figure 3A illustrates this.

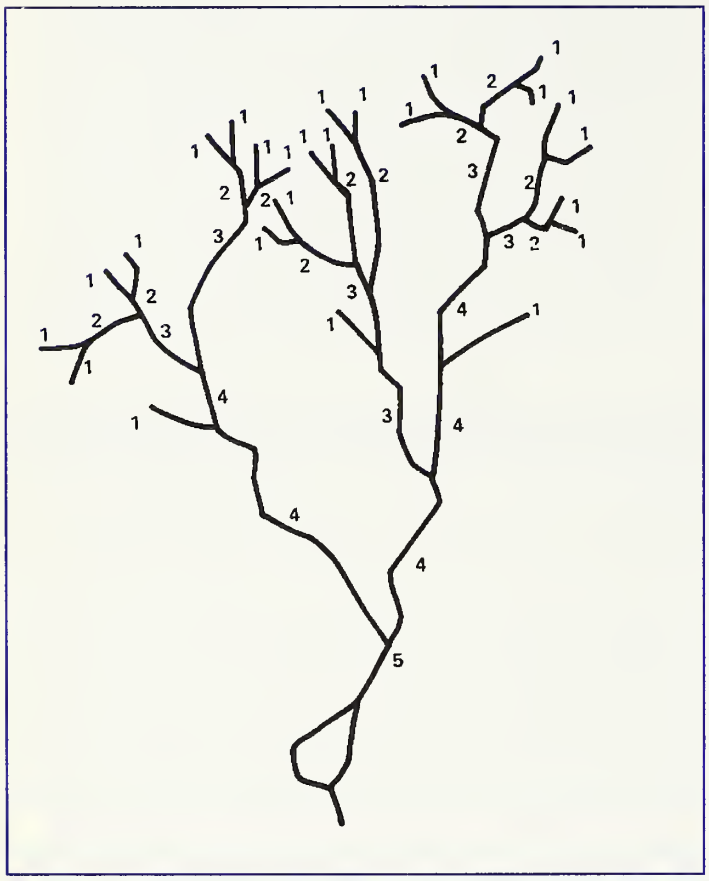

FIGURE 6

Illustration of a method of centripetal ordering applied to a Purkinje neuron (Strahler's method). All terminal dendritic segments are designated as first-order branches (1°). The ordering sequence begins with the 1° branches and proceeds toward the cell body (centripetally). Each dendritic junction in the path toward the cell body is formed by three branches. If two distal branches are from the same order (e.g., 1° branches), the third branch (proximal to the cell body) is placed in the next highest order (e.g., second-order branch, 2°). If two distal branches are not the same order (e.g., 1° and 2°), there is no change in order. The third proximal branch extends toward the cell body as a continuation of the higher order branch (e.g., 2°). In this system of ordering, any branch of order >1 may be composed of more than one segment or link. Reproduced with permission from Pentney (1986).

Golgi preparations provide a powerful tool for studying quantitative relations within individual neurons and for identifying specific and/or preferred sites of change within the networks. An example of a procedure for measuring the topology of the branching elements in a Purkinje dendritic network according to centripetal orders of branching is shown in figure 6. Measurements of the topology of dendritic branching, the area of the network, and the distribution of dendritic length within networks of Golgi-impregnated neurons have provided information about the effects of ethanol on neurons.

Dendrites of Purkinje Neurons

From measurements of Golgi-Cox impregnated Purkinje neurons, neurons in young rats treated with ethanol for 10 weeks between 3 and 5 months of age showed no change in the number of branching elements in the Purkinje cell networks. In contrast, a significant reduction in the number of terminal (first order) branches and next-to-terminal branches in the periphery of the dendritic networks was observed in neurons of older rats given the same treatment

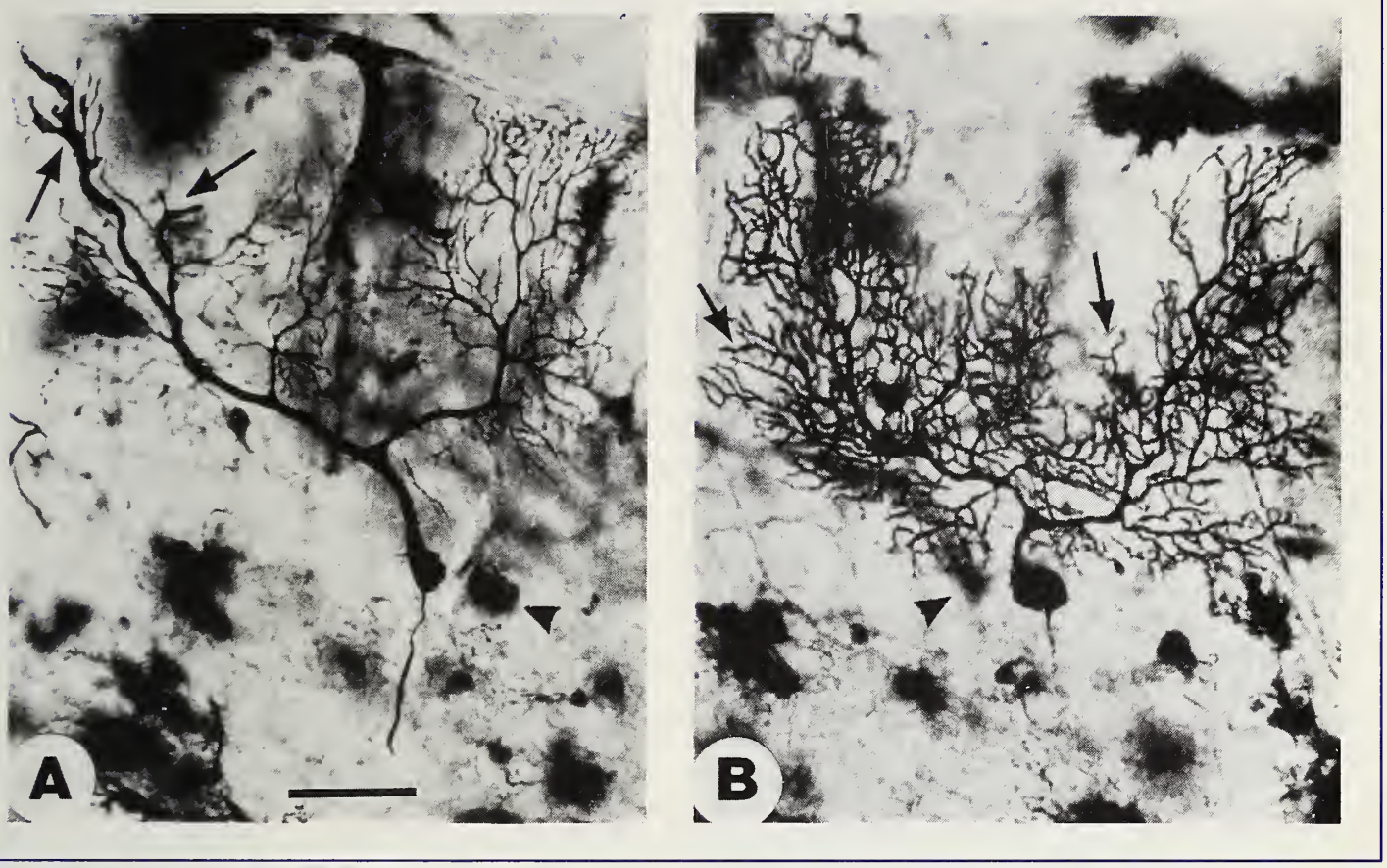

FIGURE 7

Photomicrographs of two neighboring Purkinje neurons in a 24-month-old rat fed an ethanol diet for 48 weeks. The neuron in A showed an unusually severe type of regression seen only in neurons of ethanol-treated rats in this study. The soma and all of the spiny branchlets were greatly reduced in diameter. In A and B, each arrowhead points to the shadow of the soma of the neuron focused in the adjacent frame. The dendritic network in B appeared to be depleted of some dendritic segments but mainly in the mid-upper half of the network. Arrows point to examples of swollen, stumpy terminal dendritic segments. Calibration bar: 50 μm. Reproduced with permission from Pentney and Quackenbush (1990).

between 12 and 14 months of age (Pentney 1982). This resulted in neurons with smaller dendritic networks and reduced afferent inputs. These results suggested that even relatively short terms of chronic ethanol intake induced structural changes within the nervous system that were likely to result in functional decline. The age of the subject was also an important factor in determining the occurrence and extent of that damage. In 24-month-old rats, severe regression of Purkinje neurons was occasionally observed after 12 months of ethanol treatment (figure 7) (Pentney and Quackenbush 1990).

Similarly, Tavares et al. (1983*a*) found that, when 2-month-old rats were given aqueous ethanol for 1, 3, 6, 12, or 18 months, 3 months of treatment was sufficient to initiate a subsequent steady decline in network parameters. The total length of the dendritic network declined after 3 months, the network area was reduced after 6 months, and the density of dendritic branching decreased after 12 months (figure 8). Walker et al. (1980) also reported a significant 25- to 30-per-

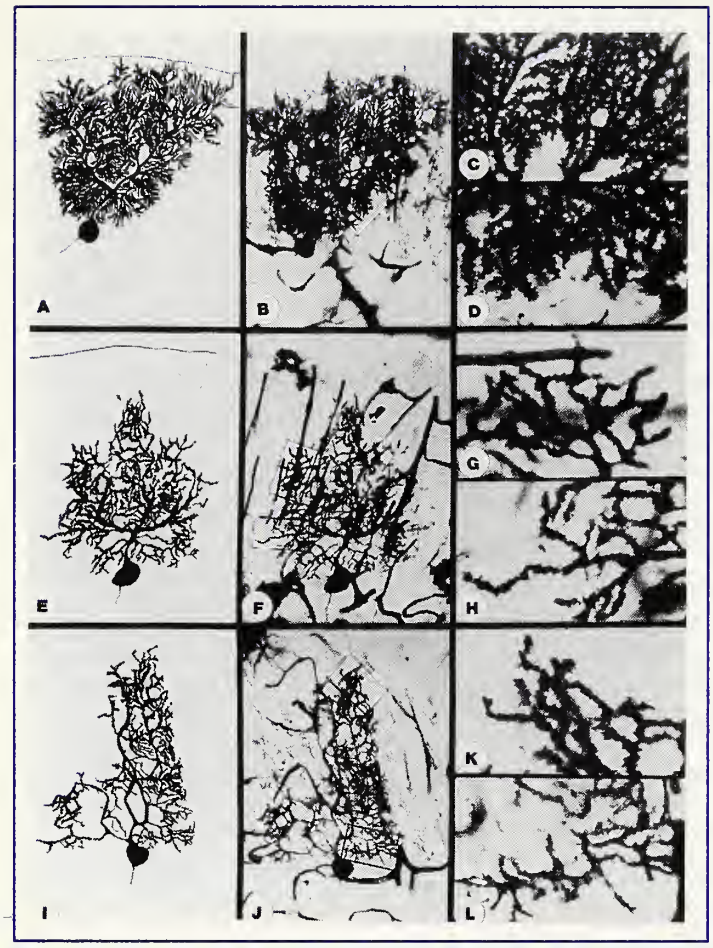

FIGURE 8

A-B, Camera lucida drawing and corresponding photographic montage of a Purkinje cell from a matched control rat. Note dendritic field area, branching pattern, and spine density. x500. C-D, Higher magnification of apical and basal spiny branchlets from the same cell, to show normal spine appearance. x2,000. E-F, Purkinje cell from a rat fed aqueous ethanol for 6 months. There is a reduction of the dendritic field area and impoverishment of dendritic branching and spine density. x500. G-H, The reduction in spine density is seen better at a higher magnification. x2,000. I-J, Purkinje cell from a rat fed aqueous ethanol for 12 months. The changes shown in E-F are more marked in these figures. x500. K-L, Spiny branchlets are often seen without spines. x2,000. Reproduced with permission from Tavares et al. (1983*a*).

cent decrease in the area of Purkinje cell dendritic networks from young adult rats after 20 weeks of ethanol consumption.

Data from all studies agreed that an ethanol-induced decline in the size of the Purkinje cell dendritic tree had occurred. Tavares et al. (1983*a*) and Pentney (1982) further identified the terminal and next-to-terminal branches as the sites of regression, suggesting that the ethanol-associated dendritic regression proceeded in a centripetal direction, from the periphery toward the cell body of the neuron.

Since ethanol-associated dendritic regression began in the periphery of the Purkinje dendritic network, it seemed logical to expect that the underlying cause of this regression might be related to the fact that peripheral branches are farthest from the synthetic center of the cell, the cell body. Each dendritic branch is linked to the cell body internally by cytoskeletal elements that extend from the cell body through that dendrite, providing for support and transport of molecules needed for dendritic maintenance. If ethanol alters the dendritic cytoskeleton, the dendrites located farthest from the cell body would be affected most. There is evidence to support this hypothesis. Paula-Barbosa and Tavares (1985) found that 1 month of ethanol consumption was sufficient to induce a significant reduction in the number of microtubules in the Purkinje cell dendrites, a change that became more pronounced with longer durations of treatment. The dendrites used for quantitation of microtubular content were the primary dendrites, not the small-diameter, terminal branches. However, it seemed reasonable to expect that such effects should be present in the fine-diameter dendrites in the periphery of the networks.

Neuronal dendrites and their spines provide most of the receptive surface of a neuron for transfer of incoming (afferent) information. A loss of dendritic surface

due to loss of dendritic branches should entail a loss of dendritic spines as well, and Tavares et al. (1983*a*) verified a significant decline in the density of spines on Purkinje cell dendrites after 12 months of ethanol treatment.

Confirmation that the loss of spines was a valid indicator of decreased synaptic input to the Purkinje neuron was obtained from quantitative study of synapses between parallel fibers and Purkinje cell spines in electron micrographs (Tavares et al. 1987*b*). The numerical density of synapses between parallel fibers and Purkinje cell spines decreased by 18 percent in ethanol-treated rats after 6 months of treatment, and by 25 to 50 percent after 18 months of treatment. Phillips (1985) conducted a similar study in ethanol-treated mice. He was unable to detect a change in the number of synapses in the molecular layer, but the postsynaptic density in the synaptic profiles increased in length during the 4 months of ethanol treatment. This investigator also reported that, after a subsequent 4-month period of recovery, there was a significant decrease in the number of synapses in the molecular layer, a result not corroborated by other investigators, however.

It is noteworthy that the number of synapses between parallel fibers and Purkinje cell spines in the electron micrographs decreased significantly after 6 months of treatment (Tavares et al. 1987*b*), but in the Golgi-impregnated neurons, the numbers of spines decreased significantly only after 12 months of treatment (Tavares et al. 1983*a*). These facts suggest that the Purkinje cell spines may persist for some time after being functionally deprived of their parallel fiber afferents. Since the parallel fibers afferent to the Golgi-impregnated neurons are not themselves impregnated, it was not possible to distinguish deafferented spines from functional spines in the Golgi material.

Change in Axons: Parallel Fibers

Consistent with the ethanol-associated loss of granule cells described above, there was evidence of degenerating parallel fibers in the molecular layer after 6 months of ethanol treatment (Tavares et al. 1983*b*).

Overview of Regressive Changes in Neuronal Processes

From the results presented above, we find two types of evidence in support of the hypothesis that regressive changes in neuronal structure stemmed from direct neurotoxic effects of ethanol on structural entities intrinsic to the neurons. These are the ethanol-associated mitochondrial abnormalities observed in Purkinje neurons and the significant ethanol-induced reduction in the number of microtubules in Purkinje cell dendrites. Unfortunately, similar data are not available for the granule neuron, the neuron that seems most sensitive to ethanol treatment.

Some results presented above are also consistent with the hypothesis that much of the ethanol-induced regression observed in cerebellar neurons results from transsynaptic influences. Regression is thought to be initiated in the granule neuron. Currently, we have an insuffi-

cient explanation for the effects of ethanol in the granule neurons. Both the mossy fibers and the Golgi neurons appear unaffected by ethanol. Thus, there is no evidence to suggest that presynaptic influences are responsible. As granule neurons and their processes degenerate, the glomeruli become disrupted through granule cell dendritic regression. As the parallel fibers also degenerate, subpopulations of stellate neurons and basket neurons are deprived of most of their excitatory input (92 and 79 percent, respectively, to their cell bodies; 100 and 89 percent, respectively, to their dendrites). The extent of depletion of excitatory input to Purkinje neurons has not been quantitated, but it also might become extensive with time. Deafferented stellate neurons, basket neurons, and Purkinje neurons may subsequently regress. The chronology of cell loss in the populations of stellate neurons, basket neurons, and Purkinje neurons appears to correlate with the relative dependency of each neuronal type on parallel fiber input. It is noteworthy, however, that parallel fibers also innervate Golgi neuron dendrites in the molecular layer, but Golgi neurons do not show any detectable changes in their structure because of parallel fiber regression.

ETHANOL-RELATED NEURONAL HYPERTROPHY

Current evidence now suggests that all rat cerebellar neurons that survive 12 months of aqueous ethanol treatment will subsequently show some evidence of hypertrophy. This may be a type of recovery within an injured neuron or a type of compensatory growth responding to ethanol-induced damage in neighboring neurons. So far, we know that ethanol-associated hypertrophy may occur in parallel fibers of granule neurons, in dendrites of stellate, basket, and Golgi interneurons, and in dendrites of Purkinje neurons. Furthermore, climbing fibers also undergo hypertrophy, but mossy fibers do not.

Parallel Fibers

Studies of electron micrographs of the rat cerebellar cortex provided evidence of parallel fiber degeneration in the molecular layer, resulting from granule neuron regression, after 6 months of treatment with aqueous ethanol (Tavares et al. 1983*a*). After 18 months of such treatment, the more prominent change observed in the molecular layer of the ethanol-fed rats was enlargement of varicose swellings of the parallel fibers, known sites of synapses with Purkinje neurons and interneurons. The volume density of the varicose swellings was significantly increased as a direct effect of the lengthier treatment. The increase in volume density was due entirely to an increase in size of individual varicose swellings, since there was no increase in the number of varicosities. Evidence that enlargement of varicosities was in progress appeared in some rats after 12 months of treatment, but the increase in volume density of the varicosities reached significant levels in the entire group of rats only after 18 months of ethanol treatment (Tavares et al. 1986). The fact that a significant loss of granule

neurons occurred 12 months before significant hypertrophy of surviving parallel fibers was achieved suggested that extensive degeneration of parallel fibers was required before extensive plasticity occurred in the parallel fiber system.

A compensatory increase in the size of axonal processes necessitates an increase in their content of microtubules. Both qualitative and quantitative appraisals of the microtubular component of parallel fibers in ethanol-treated rats indicated that the number of microtubules in parallel fibers increased progressively during 3 to 18 months of ethanol intake (Paula-Barbosa and Tavares 1985). These results contrast markedly with the ethanol-induced decrease in the number of microtubules in Purkinje dendrites during the same treatment, suggesting that in the same group of rats entirely different reactive processes occurred in dendrites and axons.

Climbing Fibers

The imbalance in Purkinje cell inputs created by the loss of granule neurons appeared to induce a reactive state in the climbing fibers that also innervate the Purkinje neurons. From a study of electron micrographs, a subpopulation of Purkinje dendritic spines on the shafts of the large diameter dendrites, normally in contact with climbing fibers, was not associated with presynaptic structures after 12 months of ethanol treatment. There was no apparent change in the number of these spines, but some spines lacked presynaptic contacts and appeared surrounded by glial processes. The spines were not in contact with other neuronal components. The climbing fibers, on the other hand, had established synaptic contacts with spines on the small diameter branches of the Purkinje neuron in the upper third of the molecular layer, outside the normal climbing fiber domain (Tavares et al. 1985*b*).

It was unclear from these observations why the climbing fibers had undergone axonal sprouting, a reaction known to occur in these axons after subtotal lesions in the inferior olivary nucleus where their cell bodies reside (Rossi et al. 1989). If they had not undergone axonal sprouting, then it was likely that they were reinnervating Purkinje cell spines deafferented because of parallel fiber degeneration. There was no evidence of degenerating climbing fibers in the micrographs, suggesting that damage to the inferior olivary nucleus had not occurred. Degenerating neurons may disappear from the CNS in a matter of hours or days (Sotelo et al. 1975), however, and may have been missed for that reason. Thus, axonal sprouting seems an unlikely explanation for the parallel fiber hypertrophy, but we cannot eliminate it completely as a possible explanation. On the other hand, the fact that climbing fiber terminals invaded the domain of the parallel fibers in the Purkinje cell networks lends support to the hypothesis that they were reacting to the imbalance of inputs created by the loss of granule neurons and their parallel fibers.

Golgi, Stellate, and Basket Interneurons

During consideration of ethanol-induced regression of granule neuron dendrites

above, it was noted that sometime after these dendrites degenerated, their vacated sites in the cerebellar glomeruli became occupied by dendrites of Golgi neurons (Tavares and Paula-Barbosa 1984). In general, we tend to consider growth, exclusive of obviously pathological conditions, as indicative of a healthy response. Consideration of the possible consequences of hypertrophy of Golgi dendrites and their substitution for granule cell dendrites illustrates that this view can be deceptive.

Under normal conditions, a mossy fiber transmits excitatory information to granule cell dendrites, and the granule neurons then relay the excitatory information upward to overlying Purkinje neurons. The patchy appearance of granule cell loss in the granular layer resulting from ethanol treatment suggests that most of the loss of granule cell dendrites occurs in a patchy distribution as well. Consequently, some glomeruli may lose all or most of their granule dendrites, and others may be unchanged or less severely altered.

When Golgi cell dendrites provide replacement postsynaptic sites for mossy fiber excitation, the normal circuitry becomes altered. The Golgi neuron has an inhibitory effect instead of an excitatory effect on its target neurons. Furthermore, the Golgi cell axon does not innervate Purkinje neurons but innervates granule neurons, depressing their responsiveness to mossy fiber activation. It is not possible, currently, to quantitate the effect of this type of change on Purkinje neuron output from the cerebellar cortex, but it is easy to understand that synaptic activity in the cerebellar cortex would be altered in two ways by this change. First, excitatory input to Purkinje cell dendrites would be partially depleted by loss of granule cell input. And second, inhibitory effects of Golgi neurons on remaining granule cell dendrites would now be abnormally enhanced, probably resulting in excessive depression of granule cell receptivity to mossy fiber stimulation.

The dendrites of stellate and basket neurons in the molecular layer also became hypertrophic after 18 months of ethanol treatment. There was a significant increase (89 percent) in the density of synapses between parallel fibers and dendrites of interneurons. These results indicated that a period of regression of established synapses between parallel fibers and Purkinje neurons during the first 12 months of ethanol treatment was followed by the formation of new synapses between parallel fibers and interneurons (Tavares et al. 1987*b*). These results show that, during chronic long-term ethanol intake, extensive remodeling of the cortical circuitry occurred in the molecular layer as well.

Purkinje Dendrites

Purkinje cell dendrites also hypertrophied during long-term chronic ethanol intake. In a series of experiments in which rats were given a liquid ethanol diet for 24 or 48 weeks, the predominant change in the Purkinje dendritic networks was dendritic elongation (Pentney et al. 1989; Pentney and Quackenbush 1990, 1991; Pentney 1991).

The design of these experiments was different from that used by all other investigators in that ethanol treatment was begun only after rats reached 12 months of age. The liquid diet used by Walker et al. (1980) was also used in these experiments. Prior results had shown that ethanol-related Purkinje cell degeneration occurred after only 10 weeks of treatment with a liquid ethanol diet in rats of this age (Pentney 1982). The dendritic networks of Purkinje neurons were analyzed topologically and measured completely. Despite the methodological differences just noted, the results obtained from these experiments were consistent with the hypertrophy reported by Tavares et al. (1983*b*, 1985*b*, 1987*b*).

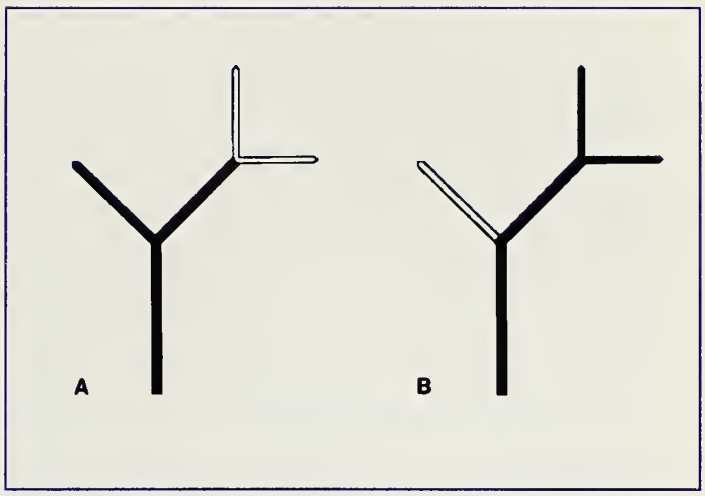

FIGURE 9

Diagram of two types of terminal dendritic segments. A, A pair of terminal segments branch from the final bifurcation point at the end of a major dendrite (outlined segments). B, Single, unpaired terminal segments branch from all other bifurcation points in the network (outlined segment). Reproduced with permission from Pentney and Quackenbush (1991).

Three significant changes in metric parameters of Purkinje dendrites were found after 48 weeks (11 months) of ethanol treatment. First, the total length of the terminal segments in networks of the ethanol-fed rats was significantly increased above control values. In contrast, there was no change in total length of the nonterminal segments. Second, the increase in total length of terminal segments developed in a nonrandom pattern. Terminal segments within these networks exist in two arrangements, as pairs of terminal segments at the tips of the dendritic shafts (approximately two-thirds of the terminal segments) and as single, unpaired terminal segments at sites along the lengths of the dendritic shafts (approximately one-third of the terminal segments) (figure 9). The mean total length of unpaired terminal segments was significantly longer in the ethanol-fed rats, whereas that of the paired terminal segments was not. Third, measurements of the two types of terminal segments showed further that the mean segment length of the unpaired terminal segments was significantly longer after the ethanol treatment.

The numbers of each type of terminal segment in the Purkinje cell networks confirmed quite convincingly that the ethanol-associated lengthening of the unpaired terminal segments was not accompanied by an increase in the number of such terminals. Unpaired terminal segments occurred in nearly equal mean numbers in controls and ethanol-fed rats. During a subsequent recovery period, there was an additional increase in total length of terminal segments, but that increase resulted from the addition of new terminal segments through branching (Pentney and Quackenbush 1990).

The spine component on the hypertrophic dendrites should be involved in any remodeling processes, since these structures provide specialized sites for synaptic contact with parallel fibers. As described above, after removal of normal parallel fiber input to Purkinje dendritic spines, the deafferented spines may be reinnervated by climbing fiber axon terminals (Tavares et al. 1985*b*), but in that situation the hypertrophic response occurred within the climbing fibers. There is other evidence, however, that suggests that the dendritic spines may also undergo hypertrophy.

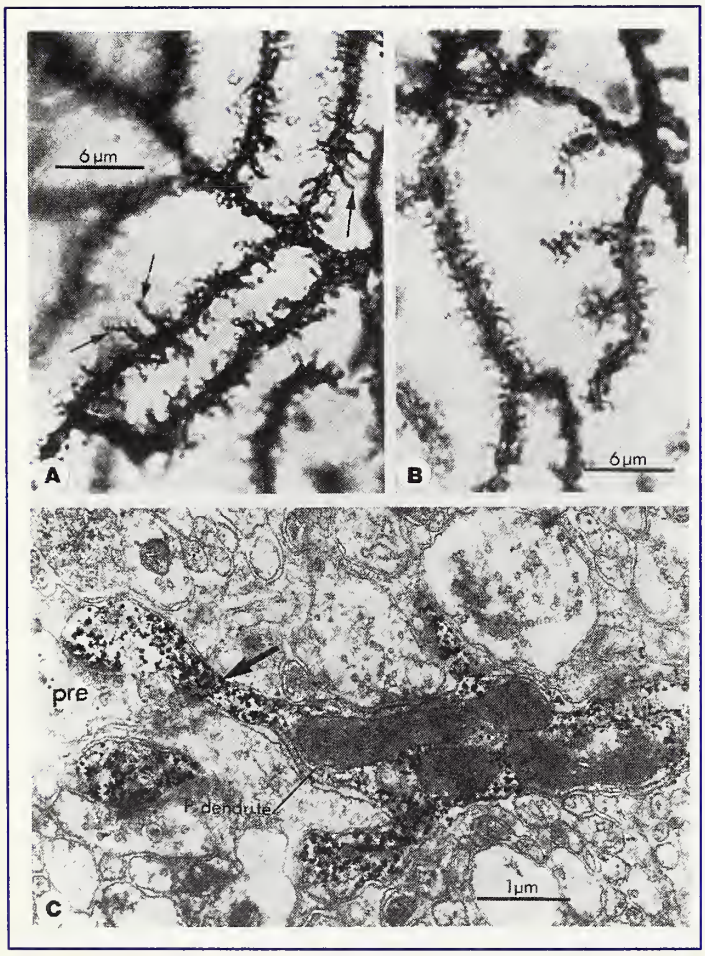

FIGURE 10

A, Elongated Purkinje dendritic spines in an ethanol-treated rat. Golgi preparation. B, Purkinje dendritic spines in a control rat. Golgi preparation. C, Golgi/EM preparation showing an impregnated, elongated Purkinje dendritic spine (arrow) from an ethanol-treated rat. Reproduced with permission from Tavares et al. (1983*b*).

After 6 months of aqueous ethanol intake, Purkinje dendritic spines decreased in total number (Tavares et al. 1983*a*), but a subpopulation of these spines increased significantly in length (Tavares et al. 1983*b*). The normal length of Purkinje dendritic spines averaged approximately 1 µm, but some hypertrophic spines were up to 3 µm long (figure 10).

Pentney and Quigley (1987) also observed unusually long dendritic spines in Purkinje neurons of ethanol-fed rats after 6 months of treatment (figure 11). In the latter study, however, it was not possible to attribute spine elongation specifically to the ethanol treatment, because elongated spines were also observed on cells from old age-matched control rats, though not as frequently as in the ethanol-fed rats. More important was the significantly increased number of spines on the terminal dendrites in the ethanol-fed rats. A complicating factor in this study was the fact that measurements were made only after the ethanol-fed rats had been allowed an 8-week recovery period. Since elongation of terminal segments, an effect related to ethanol intake and not to recovery processes, persisted throughout the recovery period, it seems likely that the spine plasticity may also have resulted from ethanol intake but persisted throughout the recovery period. Confirmation of this supposition will require more data.

CONCLUSION

Despite the incomplete status of the results obtained so far, those results show clearly that ethanol may target specific neurons

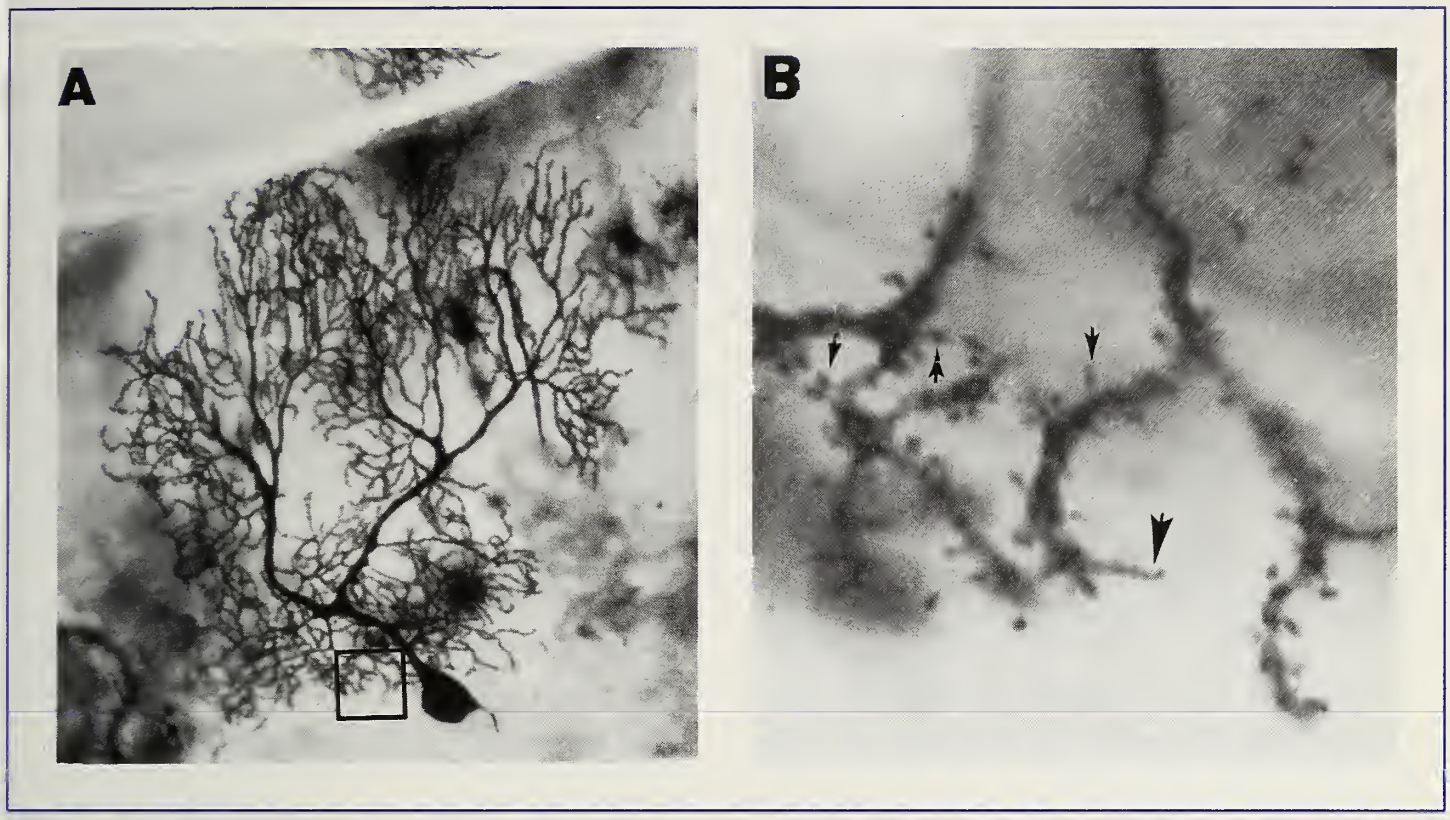

FIGURE 11

A, A Golgi-Cox-impregnated Purkinje neuron from an 11-month male rat fed a liquid diet containing 35 percent ethanol-derived calories for 6 months. Arrowheads, pial surface; framed area magnified in B, lobule VIII. x280. B, Elongated spine (large arrowhead); several spines of normal length (small arrowheads). x2,800. Reproduced with permission from Pentney and Quigley (1987).

within the cerebellar cortex, that ethanol-related structural changes may develop in those neurons, and that the effects of ethanol may be expressed in a nonrandom pattern in those neurons. As a result, the circuitry of the cerebellum may be altered in currently unpredictable ways.

In the rodent, the cerebellar cortex eventually responds to ethanol-related neuronal regression through neuronal hypertrophy and plasticity. Hypertrophy and plasticity may, however, introduce additional detrimental functional changes in cerebellar circuitry, if they produce abnormal structural interactions.

It is probable that human alcohol abuse may be associated with a similar sequence of ongoing neuronal damage and regression, followed later by compensatory hypertrophy and plasticity. Over the longer human lifespan, however, the built-in potential to compensate for neuronal regression is more likely to be overwhelmed eventually by the progress of ethanol-induced pathology. The challenge of the future is to relate structural changes identified in cerebellar neurons with specific modifications of the cerebellar circuitry and, in so doing, develop an ability to predict and understand their behavioral consequences.

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13

PHYSIOLOGICAL CHANGES ASSOCIATED WITH LONG-TERM ETHANOL CONSUMPTION

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INTRODUCTION

Neuropathological and imaging studies have emphasized pathological damage to midline diencephalic structures (Victor et al. 1971) after chronic alcohol abuse. However, neocortical damage is also a prominent feature (Lishman 1981). The relative contribution of nutritional deficiency and ethanol neurotoxicity to the mnemonic deficit has not been established (Freund 1973; Greenberg and Diamond 1985). Furthermore, the relationship between the neuropathological alterations and specific memory dysfunction has not been adequately substantiated. For example, the mammillary nuclei, which appear most consistently affected in severe cases of Korsakoff's syndrome, cannot be responsible for the associated mnemonic deficits (Mair et al. 1979; Zola-Morgan et al. 1989). Damage to the dorsomedial thalamus has also been described either alone or in combination with damage to the mammillary bodies (Butters 1985; Victor et al. 1971). Studies of amnesic patients with arterial infarction in medial thalamic territories have not clarified this issue, since the dorsomedial thalamus is not always involved (von Cramon et al. 1985). Thus, uncertainty remains over whether these diencephalic structures are important for the mnemonic deficits.

The possibility that the anterograde amnesia is related to neuronal dysfunction, not manifested by gross neuropathology, might be a likely alternative (Wilson et al. 1987). This consideration is strengthened by the fact that the diencephalic structures (mammillary nuclei, dorsomedial, anterior thalamus) most implicated in neuropathological investigations are prominent efferent projection areas of medial temporal lobe structures, which may be even more critical for memory formation (Squire 1986; Squire and Zola-Morgan 1991). Therefore, the diencephalic neuropathology may be a consequence of neuronal dysfunction that underlies the memory disturbance.

Gross neuropathological changes observed in the brains of chronic alcoholics may be insufficient in providing definitive evidence of functional alterations because the potential exists for

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compensatory processes not detectable by traditional morphological methods. For example, in the hippocampus, cell loss and deafferentation provide the stimulus for morphological reorganization (Cotman et al. 1981). Whether such morphological plasticity results in the formation of functional synaptic connections (Wilson et al. 1979) and functional recovery (Loesche and Steward 1977; Scheff and Cotman 1977) must be determined. In addition, even without morphological reorganization, surviving synaptic connections could exhibit changes in efficacy or potency that could not be detected morphologically. For example, aging results in a decrease in synaptic density in the outer molecular layer of the dentate gyrus (Geinisman and Bondareff 1976). This effect may be counteracted by an apparent compensatory increase in the potency of entorhinal afferent synaptic connections and/or an increase in the excitability of granule cells (Barnes and McNaughton 1980). Therefore, simultaneous physiological and biochemical studies are necessary to establish the nature and mechanisms underlying the neuronal dysfunction produced by ethanol.

The hippocampus has been extensively used as a model neuronal system to study a variety of disease processes including neurophysiological changes associated with chronic ethanol treatment. The hippocampus' simple (but see Amaral and Witter 1989) and highly laminated structure simplifies quantitative morphological and physiological measurements, and a wealth of background information facilitates interpretation. Numerous animal studies have confirmed that chronic ethanol administration produces abnormal morphology and function in the rodent hippocampus (see Walker et al., chapter 11). Yet despite the presence of profound changes in hippocampal morphology, chronic ethanol exposure produces surprisingly subtle changes in the function of the hippocampus as revealed by electrophysiological methods. These functional changes include modification in the distribution of afferent synaptic connections (Abraham and Hunter 1982; Abraham et al. 1982), reductions in intrinsic inhibitory processes (Abraham et al. 1981; Durand and Carlen 1984*a*), and alterations in synaptic plasticity, including long-term potentiation (Abraham et al. 1984; Durand and Carlen 1984*b*).

The purpose of this chapter is to review the literature on the functional and physiological changes in the brain produced by chronic ethanol exposure. Specifically, the chapter will focus on studies using the hippocampus as the model system and extended periods of ethanol exposure (ordinarily 3 months or longer). Details of the anatomical features of the hippocampus relevant to the understanding of electrophysiological studies are presented in another chapter (see Walker et al., chapter 11).

BASIC MEMBRANE AND SYNAPTIC RESPONSE PROPERTIES

Chronic ethanol exposure does not appear to produce dramatic changes in the properties of individual neurons in the hippocampus, as evidenced by intracellular recording techniques. Durand and Carlen

(1984*a*) have studied the properties of dentate gyrus granule cells and pyramidal cells of the CA1 region after 20 weeks of chronic ethanol treatment and a 3-week abstinence period. Prior ethanol treatment failed to alter the resting membrane potential, action potential magnitude, input resistance, time constant, rheobase current, or excitatory postsynaptic potential (EPSP) size in either cell type. However, ethanol treatment did reduce the size and temporal characteristics of the intracellularly recorded inhibitory postsynaptic potential (IPSP) and the afterhyperpolarization (AHP) that is prominent in these cell types. While such changes would be expected to increase the excitability of these cells, this has only been apparent under certain stimulus conditions. Further, since the membrane channels underlying the AHP receive convergent influence from a host of peptide and neurotransmitter receptors, the action of ethanol on this membrane parameter could result from a broad spectrum of sources (see Nicoll 1988).

Chronic ethanol effects on synaptic response properties have also been studied using extracellular recording techniques. Little or no change has been reported for the extracellular EPSP (threshold, amplitude) for Schaffer collateral/commissural (SCH/COM) afferents to CA1 in vivo (Abraham et al. 1981) or in hippocampal slices in vitro (Rogers and Hunter 1992; Rothberg and Hunter 1991). The extracellular population spike is also unaffected. This is the case for SCH/COM afferents contained in stratum radiatum or stratum oriens of CA1 (Rogers and Hunter 1992). Synaptic response properties of the extracellular EPSP, induced by activation of entorhinal afferents to the dentate gyrus, are similarly unaffected (Abraham et al. 1984). However, chronic ethanol administration did produce a relative reduction in the size of the population spike on asymptotic portions of the input/output stimulus current series (Abraham et al. 1984). These results are consistent with evidence of granule cell loss in the dentate gyrus after chronic ethanol treatment (Walker et al., chapter 11), but also implicate additional alterations in EPSP-spike coupling mechanisms in surviving cells or in action potential threshold. These latter considerations are important, since similar actions are not observed in CA1 despite comparable losses of principal cell types.

SYNAPTIC DISTRIBUTION

The distribution of synaptic currents of specific afferents can be studied with extracellular recording techniques, but the field potentials must be sampled in a spatial array orthogonal to the transmembrane current flow, a technique known as a laminar analysis. In the hippocampus the regular orientation of dendritic and cellular elements results in a summation of extracellular potentials from many of the cells. This results in large extracellular potentials in the millivolt range that are volume conducted over millimeter distances. Therefore, a simple laminar sampling of extracellular voltages lacks precision in defining areas of actual transmembrane current flow unless used in combination with mathematical

techniques such as current source density (CSD) analysis. CSD is mathematically derived from extracellular field potentials by calculation of the second spatial derivative at identical time points along the waveform. Theoretical considerations underlying this analysis as well as specific computational formulae have been presented in great detail (see Nicholson and Freeman 1975).

We investigated the persistent effect of chronic ethanol exposure on the synaptic distribution of entorhinal afferents to the molecular layer of the dentate gyrus (Abraham and Hunter 1982). Electrical stimulation of the angular bundle, which activates entorhinal afferents, revealed a short-latency, negative field potential confined to the outer two-thirds of the molecular layer. CSD analysis revealed a single, inward current sink confined to this region. Chronic ethanol administration produced a significant shrinkage of the spatial extent of the current sink in the molecular layer and a shift in the peak of the inward synaptic current toward the granule cell layer. These results suggest that chronic ethanol administration reduced the entorhinal afferent population to the molecular layer of the dentate gyrus. This action may be selective for the lateral entorhinal afferents, because they predominate in the outer portions of the molecular layer (Steward and Scoville 1976).

These conclusions, however, are difficult to reconcile based on other observations. In a recent study using Golgi staining, chronic ethanol treatment produced a decrease in the branching of proximal dendrites and an increase in branching of distal dendrites, with a corresponding increase in dendritic length (Durand et al. 1989). In another study, it was hypothesized that the increase in dendritic length after chronic ethanol was responsible for changes in specific membrane resistance and capacitance measures (Durand and Carlen 1985). One possible way to resolve these apparent inconsistencies is to suggest that the increased branching of distal dendrites, increased dendritic length, and altered dendritic electrotonic characteristics reflect a complex compensatory response to lateral entorhinal deafferentation. However, this explanation would require considerable further substantiation.

The synaptic distribution of SCH/COM afferents to stratum radiatum of CA1 also appears to be altered by chronic ethanol treatment. Stimulation of the SCH/COM afferents elicits a short-latency, negative field potential throughout the synaptic terminal zone in stratum radiatum. However, in contrast to the dentate gyrus, CSD analysis revealed that the net inward synaptic currents generated in stratum radiatum concentrate into bimodal peaks approximately 70 and 220 μm from the pyramidal cell layer. Chronic ethanol administration produced a significant reduction in the spatial extent of the current sink proximal to the cell layer and a corresponding expansion in the area and amplitude of the distal sink (Abraham et al. 1982). These dual current sinks may reflect a separation of the SCH and COM afferents to CA1. According to this hypothesis,

chronic ethanol treatment would selectively reduce the COM afferent population with a corresponding reorganization of the more distally localized SCH afferent population.

Recent research has extended these observations and revealed a surprising explanation: namely, that these differences in postsynaptic currents arise from differences in the size of corresponding presynaptic currents (i.e., afferent volleys). Studies have been completed in which the sizes of the afferent volley and the EPSP were recorded in both dorsal and hippocampal slices (Smothers and Hunter 1989). The magnitude of the afferent volley in stratum radiatum *increased* following chronic ethanol exposure. In the ventral hippocampus this increase was accompanied by little or no change in the extracellular EPSP, suggesting a compensatory reduction in synaptic response strength. However, this compensatory reaction was not observed in dorsal hippocampal slices, where the increase in afferent volley size was accompanied by a corresponding increase in EPSP magnitude.

These studies have now been repeated for afferents in stratum oriens (Rothberg et al. 1990), where a *decrease* in the size of the afferent volley has been observed after ethanol treatment. These results would appear to support the previous hypothesis based upon CSD analysis because COM afferents appear to predominate in stratum oriens (Goldowitz et al. 1979; Gottlieb and Cowan 1973; Laurberg and Sorenson 1981), whereas SCH afferents segregate in stratum radiatum.

Collectively, the results support the hypothesis that chronic ethanol exposure selectively reduces a population of afferents to CA1 arising from the contralateral hippocampus, as evident from the decrease in afferent volley in stratum oriens. An apparent compensatory response occurs in stratum radiatum, where the magnitude of the afferent volley is increased. Current evidence indicates that a gradient exists in the CA3 projections to CA1, such that projections to stratum oriens and proximal stratum radiatum tend to originate in CA3 cells bordering CA1 (Amaral and Witter 1989). However, retrograde fluorescent labeling studies have revealed populations of double-labeled CA3 pyramidal cells, which exhibit both SCH and COM afferent branches (Laurberg and Sorensen 1981). Thus, determination of whether a selective ethanol action on a subpopulation of CA3 cells can explain these findings must await additional confirmatory evidence. Nevertheless, even if the hypothesis about the source of these afferents is incorrect (i.e., COM and SCH), the results still indicate that afferents in stratum oriens are differentially affected from those in stratum radiatum after chronic ethanol treatment.

INHIBITORY SYNAPTIC FUNCTION

Because a substantial amount is known regarding the organization of inhibitory synaptic function in the hippocampus, it is an ideal model to investigate the chronic actions of ethanol on these functions. In particular, local circuit interactions

within the CA1 subfield of the hippocampus have been well characterized. In this region pyramidal cell excitability is under the control of feedforward and feedback (recurrent) inhibition.

Feedforward inhibition is mediated by direct synaptic activation of interneurons by SCH and COM afferent input. Recordings from identified cells in CA1 have shown the presence of interneurons responsive to direct afferent activation at latencies and thresholds that are actually lower than those of pyramidal cells (Ashwood et al. 1984; Lacaille et al. 1987, 1989). In fact, activation of interneurons may occur so rapidly that IPSPs induced by feedforward afferents influence the rising and falling phases of EPSPs (Turner 1990).

Morphological (Lorente de No 1934) and physiological criteria have defined three populations of interneurons. Two of these interneuronal types, located in or near the pyramidal cell layer (Lacaille et al. 1989) and at the stratum oriens/alvear border (Lacaille et al. 1987), can be activated by both CA1 pyramidal cells and SCH/COM afferents. The third interneuronal type, localized to the stratum radiatum/stratum lacunosum-moleculare border, responds only to SCH/COM input and not from CA1 pyramidal cells (Lacaille and Schwartzkroin 1988).

Paired-pulse stimulation techniques have been extensively used to characterize inhibitory function in the hippocampus. In this procedure a pair of stimulation pulses is applied to the same or different afferent populations. When an afferent is subjected to an initial conditioning stimulation pulse, the local circuitry within the hippocampus becomes activated. The nature of the influence on a succeeding test pulse depends upon the configuration of the stimulating and recording electrodes (i.e., afferents activated) and the temporal relationship between the stimulus pair.

With antidromic stimulation, the conditioning pulse is applied to the axons of the principal cell type. In CA1, the conditioning pulse is applied to the alveus, which contains axons of CA1 pyramidal cells. The result of this stimulation is an antidromic volley that invades the pyramidal cell bodies. Activation of recurrent axon collaterals also occurs, leading to activation of inhibitory interneurons. This results in powerful inhibition of the pyramidal cell discharge, which consists of two phases.

An initial phase, peaking at roughly 20 ms, can completely inhibit pyramidal cell discharge and is particularly sensitive to drugs that modify γ-aminobutyric acid ($GABA_A$) receptor function (Dunwiddie et al. 1986; Kapur et al. 1989). A decay to a more enduring phase of inhibition follows; this likely involves activation of $GABA_B$ receptors (Alger and Nicoll 1982).

A second type of paired-pulse stimulation has also been used to study inhibitory processes and involves stimulation confined to a single afferent population. Such homosynaptic stimulation, however, is more difficult to interpret, because it involves additional factors, including a facilitation of EPSP amplitude and activation of feedforward inhibitory processes as well as the recurrent pathway.

In an initial study to determine whether chronic ethanol resulted in functional changes of the Schaffer collateral/commissural afferents to rat CA1, only subtle electrophysiological changes were observed after 2 to 4 months of abstinence (Abraham et al. 1981). The major findings showed, paradoxically, that chronic ethanol increased pyramidal cell excitability in condition/test pulse paradigms and in response to low frequency (5 to 10 Hz) stimulation. Because similar results had been reported under conditions in which synaptic inhibition is reduced (Dunwiddie et al. 1980), it was hypothesized that these data reflected a decreased efficacy of recurrent inhibitory processes in the CA1 region. However, these studies were undertaken in urethane–anesthetized animals, creating a potential confounding interaction of anesthesia with prior ethanol exposure.

Recent investigations addressed these issues in hippocampal slices (Rogers and Hunter 1992) and involved both antidromic and homosynaptic paired-pulse stimulation. Chronic ethanol exposure produced a significant reduction in recurrent inhibition, as assessed by antidromic conditioning stimulation. This reduction was most apparent at high conditioning stimulus intensities and could have been mediated by current spread to adjacent feedforward afferents in stratum oriens. However, whether chronic ethanol exposure also produced a disruption of feedforward inhibition was not resolved by these studies. It seems unlikely that a change in recurrent inhibition would occur without a change in feedforward inhibition because interneurons responsive to alvear stimulation are typically responsive to Schaffer collateral/commissural activation (Lacaille et al. 1989). Additional research is required to address this important issue.

These results indicate that chronic ethanol exposure produces an enduring disruption of synaptic inhibition in the CA1 of the hippocampus. This conclusion is supported by intracellular recordings in CA1 pyramidal cells (Durand and Carlen 1984*a*). Similar results were observed for granule cells of the dentate gyrus. We currently do not know if other areas of the brain are similarly affected. There are few interneurons in the hippocampus and their axons ramify extensively. Therefore, disruption in the function of even a few cells could have a major impact on the function of the hippocampus. The site and mechanism of chronic ethanol's action are not clear. Chronic ethanol decreases the number of CA1 pyramidal neurons (Walker et al., chapter 11), and this reduction in inhibition could merely reflect a loss of input from afferent recurrent axon collaterals to inhibitory interneurons. Alternatively, chronic ethanol exposure could reduce the number of interneurons. Immunocytochemical studies have shown that chronic ethanol treatment reduces the number of GABA immunoreactive interneurons in the CA1 of the mouse hippocampus (Lescaudron et al. 1986). The reduction in inhibition could also result from changes at GABAergic synapses. Intracellular recordings in CA1 pyramidal neurons have shown a significant

reduction in the amplitude of spontaneously occurring IPSPs following chronic ethanol exposure (Durand and Carlen 1984*a*). This reduction in IPSP could reflect presynaptic or postsynaptic changes in GABAergic function.

We have completed the analysis of a series of studies in hippocampal slices designed to evaluate the mechanisms underlying the effects of chronic ethanol exposure on inhibition. We examined population spike responses evoked by stimulating stratum radiatum in response to iontophoretic administration of GABA, bicuculline (a specific $GABA_A$-receptor antagonist), and baclofen (a specific $GABA_B$-receptor agonist) at points in stratum radiatum, stratum oriens, and in the pyramidal cell layer (Rogers and Hunter submitted).

GABA has been shown to affect pyramidal cell excitability in several ways. With local application to the pyramidal cell layer, GABA produces a powerful hyperpolarizing IPSP. When GABA is applied to pyramidal cell dendrites, a depolarizing IPSP, which appears to act by "shunting" excitatory synaptic current flow, is also observed (Anderson et al. 1980). Both the somatic hyperpolarizing and dendritic depolarizing IPSPs are antagonized by bicuculline (Alger and Nicoll 1982; Anderson et al. 1980). GABA also produces a slower, late-hyperpolarizing IPSP when applied to the apical dendrites (Alger and Nicoll 1982). This later-hyperpolarizing IPSP is bicuculline resistant, may act by increasing a potassium conductance, can be mimicked by application of baclofen, and can be blocked by saclofen and other specific $GABA_B$-receptor antagonists (Andrade et al. 1986; Dutar and Nicoll 1988; Harrison et al. 1990; Lambert et al. 1989).

The sensitivity to GABA was evaluated by iontophoretic application at 10 equidistant points parallel to the dendritic axis of pyramidal cells in CA1 from the alveus to the hippocampal fissure (Rogers and Hunter submitted). Drug concentrations, ejection durations, and backing currents were derived from prior studies (Anderson et al. 1980). GABA application to the pyramidal cell layer produces a reduction in evoked population spikes in stratum radiatum, extending throughout most of stratum radiatum. Chronic ethanol treatment failed to influence this inhibitory action of GABA. When bicuculline was iontophoretically ejected under similar conditions, a profound increase in population spikes was observed. This increase often involved the production of multiple population spikes, peaking at approximately 1 minute after iontophoretic administration, and was greatest in magnitude with sites located close to the pyramidal cell layer. Chronic ethanol exposure produced a surprising increase in the excitatory action of bicuculline that was most prominent with application to stratum oriens and near the pyramidal cell layer. Based on these results, the chronic ethanol-induced reduction in inhibitory synaptic function (Rogers and Hunter 1992; Durand and Carlen 1984*a*) apparently cannot be explained by a reduction in postsynaptic $GABA_A$ receptor function. The increase in the sensitivity to bicuculline could best be

explained by a decrease in presynaptic GABA release. The current working hypothesis is that this reduction in GABA release involves a decrease in GABAergic interneurons (Lescaudron et al. 1986), although enduring changes in synaptic terminal number or excitability or GABA release cannot be ruled out.

A possible role for $GABA_B$ receptors in mediating the reduction in inhibition produced by chronic ethanol treatment must also be evaluated. We have examined changes in sensitivity to the $GABA_B$ agonist baclofen in a paradigm identical to that described for bicuculline. Iontophoretic administration of baclofen produced a site-dependent inhibition of population spike amplitude that developed slowly, peaked at approximately 1 to 2 minutes postejection, and decayed over a protracted time course. The greatest inhibition of population spike amplitude was produced by ejecting the drug in stratum radiatum in the trajectory of the stimulating electrode. Ejection of baclofen in the pyramidal cell layer and stratum oriens resulted in significantly less inhibition. The response to baclofen was essentially unchanged as a function of prior ethanol treatment. These results argue against a role for $GABA_B$ receptors in the reduction in inhibition produced by chronic ethanol treatment.

However, in addition to the postsynaptic $GABA_B$ receptors present on the dendrites of pyramidal cells, presynaptic $GABA_B$ receptors also exist and act to inhibit transmitter release (Dutar and Nicoll 1988). This fact could explain why the greatest inhibition observed in our studies with baclofen was in the synaptic terminal region in stratum radiatum. By measuring population spikes, both presynaptic and postsynaptic $GABA_B$ receptors would be activated and a selective action of prior chronic ethanol exposure on one subtype could have been masked. Such selectivity is potentially very important. Frye et al. (1991) have found that acute ethanol treatment failed to alter responses by either presynaptic or postsynaptic $GABA_B$ receptors. However, after 12 days of exposure a significant reduction in the $GABA_B$ postsynaptic response was observed, whereas no change in the presynaptic component was observed. It should be noted that these responses were assessed in hippocampal slices obtained from animals without ethanol removal and at a shorter duration of exposure. Therefore, it is not clear whether enduring changes would persist after chronic ethanol exposure. Nevertheless, a separation of responses of these receptor subtypes will be necessary before the role of hippocampal $GABA_B$ receptors and chronic ethanol exposure can be definitively determined.

CHOLINERGIC SYNAPTIC FUNCTION

In view of the well-established link between cholinergic synaptic function and memory in animal models and in humans, the possible role of the septohippocampal cholinergic pathway in mediating the deleterious actions of chronic ethanol exposure must be considered. Chronic alcoholism is accompanied by a degeneration of the cholinergic neurons in

the basal forebrain and a decrease in the activity of choline acetyltransferase in the cortex and hippocampus (Antuono et al. 1980; Nordberg et al. 1983; also see Arendt, chapter 22). Arendt and coworkers have shown that chronic ethanol in rats produces a loss of acetylcholinesterase-positive neurons in the basal forebrain, a decrease in acetylcholine concentrations, and a reduction in the activity of choline acetyltransferase in the basal forebrain and hippocampus (Arendt et al. 1988, 1989). Chronic ethanol treatment also produced a progressive decline in spatial memory over 4 to 28 weeks, paralleled by a decrease in the cholinergic parameters in both basal forebrain and hippocampus. These studies are of considerable significance in providing an important link between atrophy of hippocampal and basal forebrain neurons, septohippocampal cholinergic dysfunction, and impaired memory. However, it is unknown if septohippocampal cholinergic dysfunction is accompanied by changes in cholinergic synaptic function in the hippocampus. We have begun to address this issue by studying changes in the responses of cholinergic synapses after chronic ethanol administration (Rothberg and Hunter 1991).

The cholinergic afferent projections to the hippocampus have been well characterized. The source of these afferents lies in the basal forebrain, where several nuclear subdivisions of cholinergic projections have been identified. These nuclear groups include the (1) medial septal nucleus and the nucleus of the vertical limb of the diagonal band of Broca, which project to the hippocampus; (2) nucleus of the horizontal limb of the diagonal band, which projects to the olfactory bulb, and pyriform and entorhinal cortex; and (3) nucleus basalis, which projects to the neocortex and amygdala (Houser et al. 1983; McKinney et al. 1983; Mesulam et al. 1983; Matthews et al. 1987). The intrahippocampal distribution of cholinergic afferents is quite unlike many hippocampal afferents that terminate in highly laminated, discrete terminal zones on the dendrites of granule or pyramidal cells. Immunocytochemical studies of choline acetyltransferase have localized concentrations of cholinergic afferents near the pyramidal and granule cell layers, but small numbers of afferents appear to invade all layers of the dentate gyrus and hippocampus proper (Matthews et al. 1987). Convincing evidence of cholinergic synaptic contacts on all principal and interneuronal cell types has been demonstrated (Frotscher 1988).

The physiological properties of acetylcholine in the hippocampus have been extensively characterized. Application of acetylcholine to CA1 or dentate gyrus leads to a slow EPSP that is associated with a membrane depolarization and reduction of AHP (Brunner and Misgeld 1988; Madison et al. 1987). Voltage clamp studies have shown these effects to be mediated by *closing* three or more types of voltage-dependent potassium channels, including a K^+-leak current (representing channels open at the resting membrane potential), the M-current (representing muscarinic-inactivated channels), and a KCa-current (representing calcium-dependent chan-

nels), which is probably most responsible for the enduring AHP (Madison et al. 1987). Muscarinic receptors mediate each of these actions, because they can be blocked by atropine but not by nicotinic antagonists. With extracellular recordings, acetylcholine application to pyramidal cells leads to an increase in discharge or population spike amplitude in accord with the slow muscarinic depolarization found intracellularly (Ropert and Krnjevic 1982; Rovira et al. 1983; Valentino and Dingledine 1981). In contrast, application in the synaptic zone in stratum radiatum leads to depression of the extracellular EPSP and associated population spike (Hounsgaard 1978; Valentino and Dingledine 1981). These latter actions suggest the presence of presynaptic receptors on the terminals of stratum radiatum afferents that reduce transmitter release. These effects are also blocked by atropine, but not hexamethonium (a nicotinic antagonist), indicating a muscarinic action.

Cholinergic synaptic function has been studied by examining the sensitivity of population spikes and dendritic EPSPs to local, iontophoretic application of acetylcholine in hippocampal slices at 5 to 6 months following chronic ethanol exposure (Rothberg and Hunter 1991). Prior chronic ethanol exposure was found to significantly reduce the facilitation of population spike amplitude observed up to 10 to 20 seconds following acetylcholine ejection. In contrast, the reduction of the dendritic EPSP induced by focal application of acetylcholine was unchanged by prior chronic ethanol administration. However, a role for ethanol-induced changes in acetylcholinesterase or nicotine receptors cannot be dismissed because acetylcholine was used in these studies. This possibility is unlikely because changes in acetylcholinesterase would likely alter the time-course of the response. In addition, nicotine receptor activation does not contribute to these specific cholinergic responses. The reduction in muscarinic receptor function also stands in contrast to results of fimbria-fornix transection studies, where a functional supersensitivity in hippocampal slices has been characterized (Benson et al. 1989). These differences point to the importance of the temporal characteristics of the cholinergic deafferentation in determining the ultimate neuronal response to injury.

Chronic ethanol exposure may produce an enduring reduction in muscarinic receptor function, but not all muscarinic receptor responses are affected. The use of selective muscarinic antagonists in combination with Schild plot analysis has revealed that the population spike and dendritic EPSP may be mediated by pharmacologically distinct muscarinic receptor subtypes (Pitler and Alger 1990; Sheridan and Sutor 1990). At least five distinct muscarinic receptor subtypes have now been characterized, and each has been measured after chronic ethanol exposure using immunoprecipitation assays (see Wall et al. 1991).

Although preliminary, the results suggest that chronic ethanol treatment fails to alter the concentration of any specific muscarinic receptor subtype in the hippocampus (Rothberg et al. 1992).

Therefore, chronic ethanol may act to alter processes downstream from the muscarinic receptor itself such as at the second messenger or ion channel level. Thus, there may be a relation between the acute actions of ethanol and more enduring, chronic actions.

Acute ethanol exposure significantly enhances excitatory responses of pyramidal cells to acetylcholine by a mechanism believed to involve a synergistic action of acetylcholine and ethanol on the M-current, a voltage-dependent potassium channel modulated by a variety of transmitter receptors (Moore et al. 1990). Our results are consistent with an adaptive (downregulation) response at this effector, but such adaptation must be maintained over prolonged periods (i.e., 5-month abstinence). Similarly, chronic ethanol treatment has been shown to reduce an enduring AHP in pyramidal cells (Durand and Carlen 1984*a*). The slow AHP is in large part mediated by a KCa, which also receives convergent modulation from a variety of transmitter receptors including muscarinic receptors (Madison et al. 1987). Substantial additional evidence will be required to evaluate the potential role of these membrane ion channels in this effect of chronic ethanol treatment.

SYNAPTIC PLASTICITY: LONG-TERM POTENTIATION

Long-term potentiation (LTP) has been an intensively studied physiological model of long-term memory formation. As originally conceived, LTP was defined as an enduring change in synaptic strength occurring because of high-frequency stimulation (Bliss and Lomo 1973). This change, which can last from hours to days (Bliss and Lomo 1973; Gustafsson and Wigstrom 1988; Teyler and DiScenna 1985) is manifested by an increase in the amplitude and the slope of the extracellular recorded EPSP. LTP also results in an increased amplitude and decreased latency of the population spike. These changes are also observed in individual neurons using intracellular recording techniques (Gustafsson et al. 1987). At the physiological level, the induction of LTP requires cooperativity of synaptic inputs resulting in a threshold depolarization of the postsynaptic membrane. With extracellular stimulation, LTP occurs following high frequency stimulation of afferent fibers sufficient to produce this threshold level of depolarization (McNaughton et al. 1978). At the intracellular level, depolarizing currents of sufficient magnitude produce a similar cooperative action (Gustafsson et al. 1987). However, because depolarizing the postsynaptic membrane is not sufficient to produce potentiation, such LTP requires a coupling of presynaptic transmitter release with the postsynaptic depolarization.

The molecular mechanisms important in the induction of the synaptic component of LTP in the CA1 region have been extensively characterized. The trigger for the induction of LTP is at the N-methyl-D-aspartate (NMDA) receptor/ion channel complex. NMDA receptors are apparently unique in that they require both postsynaptic glutamate binding in combination with sufficient postsynaptic depolarization to allow relief of a voltage-

dependent Mg^{2+} block of the NMDA receptor/ion channel (Collingridge and Bliss 1987; Dingledine 1983; Kauer et al. 1988; Malenka et al. 1988; Nowak et al. 1984; Thibault et al. 1989). The NMDA receptor is coupled to a nonspecific cation channel that allows significant transmembrane Ca^{2+} flux. The Ca^{2+} transient is believed to be localized exclusively in dendritic spines (Kennedy 1989; Nicoll et al. 1988; Wickens 1988) and may trigger one or more calcium-dependent enzymes such as calcium/calmodulin kinase II, protein kinase C, or proteases (Kennedy 1989; Malenka et al. 1989; Malinow et al. 1988). Current evidence suggests that these enzymes initiate a secondary cascade involving additional enzymes, including tyrosine kinase and microtubule-activated protein kinase. Ultimately, this cascade leads to the formation of diffusible substances that could serve as a retrograde messenger on the presynaptic terminal (see O'Dell et al. 1991).

Although the NMDA receptor serves as a critical trigger in the induction of LTP, alterations in NMDA receptor function appear to play little role in the expression of established LTP (but see Bashir et al. 1991). Considerable controversy exists over the mechanism and site involved in the expression of LTP (Bekkers and Stevens 1990; Kauer et al. 1988; Malinow and Tsien 1990; Manabe et al. 1992).

It seems probable that LTP expression will eventually be shown to involve both postsynaptic and presynaptic sites with differing temporal properties characterizing their onset and decay. It is also noteworthy that LTP can occur at sites where NMDA receptors do not exist (Johnston et al. 1992). Nevertheless, although NMDA receptors reach highest density in the hippocampus (Cotman and Monaghan 1988), their widespread distribution in the nervous system suggests that this form of synaptic potentiation is a significant function of many synapses throughout the brain.

Initial experiments indicated that acute ethanol administration suppresses the induction of LTP, but at relatively high concentrations (see Sinclair and Lo 1986). However, Blitzer et al. (1990) found that ethanol in concentrations as low as 30 mM almost completely abolished LTP of the dendritic EPSP in the CA1 region. This action occurred in the presence of 10 μM picrotoxin, suggesting that potential ethanol actions on GABAergic inhibition could not account for the changes in LTP. In voltage-clamped cells, a high correlation was found between the degree of LTP reduction and ethanol-induced suppression of NMDA currents. These data provide an important link between the now well-characterized sensitivity of the NMDA receptor complex to ethanol (Lovinger et al. 1990; White et al. 1990) and a reduction in LTP.

Chronic ethanol exposure also appears to reduce LTP in the hippocampus. Chronic ethanol exposure has been shown to decrease LTP of the population spike evoked by perforant path input to the dentate gyrus (Abraham et al. 1984), reduce the number of slices exhibiting LTP (Durand and Carlen 1984*b*), and produce a trend toward greater decay of LTP in CA1 (Abraham et al. 1981). However,

these studies have analyzed only the population spike component and not the synaptic component of LTP. Considerable evidence indicates that potentiation of the population spike can be distinct from potentiation of the synaptic response. For example, while LTP of the population EPSP is associated with an enhancement of the intradendritic EPSP, LTP of the population spike can occur without an associated synaptic enhancement (Taube and Schwartzkroin 1988). This feature of LTP of the population spike, which has become known as E-S potentiation (Abraham et al. 1987; Chavez-Noriega et al. 1990), may involve heterosynaptic mechanisms, including a long-term reduction in inhibitory processes (Abraham et al. 1985; Chavez-Noriega et al. 1990).

Because of the complexities of these interactions, we have recently examined the effect of chronic ethanol exposure on potentiation of the synaptic component of LTP (Tremwel and Hunter 1991). We used a procedure in which graded increases in the duration of the LTP conditioning trains (30, 40, 50, 60, 70 pulses at 100 Hz) produced incremental increases in the size of LTP. This protocol was intended to differentiate the effect of chronic ethanol on the threshold response from the maximal magnitude or asymptote of LTP. The 30-pulse train produced threshold LTP measured 10 minutes following the stimulus train, with progressively greater LTP induced by longer conditioning trains (40 to 70 pulses). Hippocampal slices from chronic ethanol-treated animals (7 months), studied after a 5- to 6-month abstinence period, were found to exhibit significant reductions in the induction of LTP. However, when slices were treated with bicuculline (5 μM), this disruption of LTP was eliminated.

LTP induction in the presence of $GABA_A$ antagonists is mediated largely by NMDA receptors. There is growing recognition that the induction of NMDA-mediated LTP can be modulated by a variety of cellular processes. Under ordinary conditions it is likely that GABAergic inhibition can modulate even the induction of the synaptic component of LTP (see Davies et al. 1991; Mott and Lewis 1991). The results of our studies suggest that chronic ethanol-induced disruption of LTP is not mediated by alterations of an NMDA component of LTP, but by some yet unspecified alteration in GABAergic inhibitory processes. In view of the growing consensus regarding a role for the hippocampus in normal memory formation (Squire and Zola-Morgan 1991), these results could provide a significant mechanism underlying the mnemonic deficits associated with chronic alcoholism. We are unaware of any other degenerative condition that produces this dissociation between NMDA and inhibitory components of LTP, and we are currently examining the temporal characteristics of its development by studying shorter abstinence periods.

CONCLUSIONS

The foregoing evidence provides ample support and justification for using electrophysiological techniques to characterize ethanol-induced brain damage. Despite

the well-characterized morphological abnormalities found in many brain regions (Walker et al., chapter 11), the physiological and functional changes produced by chronic ethanol exposure are surprisingly subtle and exhibit a great deal of specificity. Basic membrane properties are largely normal in the cell types examined thus far, and most basic synaptic responses are unchanged. Specificity appears to be exhibited for the chronic ethanol-induced changes in synaptic responses of individual pathways (stratum oriens versus stratum radiatum), individual neurotransmitters (GABA, acetylcholine), and neurotransmitter receptor subtypes (muscarinic). Even individual muscarinic receptor responses are not all equally affected by prior chronic ethanol exposure. This specificity may arise from the compensatory adaptation possible in the brain, in general, and in the hippocampus, in particular.

Perhaps the best example of evidence of simultaneous destructive and compensatory responses to chronic ethanol is in CA1, where an apparent reduction in synaptic responses in stratum oriens was paralleled by an increase in synaptic response strength in stratum radiatum. These changes in synaptic response strength appear to be linked to some alteration in CA3 pyramidal cells. Each change could be accounted for by alterations in the magnitude of presynaptic fiber volley, presumably reflected by changes in terminal branches or numbers of synapses.

However, whether the increased afferent volley is truly a compensatory response remains to be clarified. If the results obtained, to date, turn out to reflect a reduction in commissural afferent synaptic influences and an augmentation in ipsilateral, associational afferent synaptic influences, then CA1 pyramidal cells after chronic ethanol exposure may be less able to respond to information derived from the contralateral hippocampus. This bias in favor of information processed on the same side of the brain could ultimately underlie behavioral dysfunction and not serve a compensatory function at all. Thus, any apparent compensatory action produced by chronic ethanol must be carefully considered for its effect on brain function and behavior.

The relationship between the acute actions of ethanol, ethanol tolerance/physical dependence, and the chronic actions of ethanol described above is presently unclear. Most studies examining changes in neuronal function accompanying the development of ethanol tolerance and physical dependence typically observe return to control levels in a matter of days after removal of ethanol. Longer durations of ethanol exposure may be needed to produce adaptive changes that will also persist for prolonged periods of ethanol abstinence. Adaptive changes may also occur by virtue of unique attributes of the neuronal circuitry and how it is influenced by ethanol over prolonged periods, rather than by direct ethanol interactions with receptor molecules. This could help explain the paradoxical finding of a decrease in $GABA_B$ receptor responses that developed over a 12-day period, although no acute action of ethanol on

$GABA_B$ receptor function could be demonstrated (Frye et al. 1991). Defining the mechanisms underlying the chronic actions of ethanol is further complicated by the destructive and potential compensatory changes in the brain that have been found. The resulting neuronal reaction to destructive changes may preempt ordinary adaptive responses that might occur with continued exposure to ethanol.

Further studies are required to characterize the mechanisms underlying these varied actions of chronic ethanol treatment. In addition, careful parametric studies will be required to establish a link between the acute, intoxicating actions of ethanol and these chronic, relatively permanent changes in neuronal function. Such studies will need to incorporate responses measured over different durations of ethanol exposure and ethanol abstinence. Finally, since nearly all of the research on the functional changes produced by chronic ethanol exposure has used the hippocampus as a model system, it is imperative that future work begins to address these questions in other brain areas sensitive to ethanol, including the cerebellum and neocortex.

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14

RECEPTOR CHANGES ASSOCIATED WITH ETHANOL-INDUCED BRAIN DAMAGE

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INTRODUCTION

The traditional view of ethanol-induced brain damage emphasizes macroscopic and/or microscopic pathological changes in brain tissue. However, significant impairment of brain function due to ethanol clearly occurs without, or before, the appearance of histological evidence of damage. Therefore, as a possible mechanism of this biological impairment, the broad question of receptor changes associated with ethanol-induced impairment will be the focus of this chapter. A distinction will be made between those receptor changes thought to mediate ethanol-induced brain impairment, and those likely to be the consequence of ethanol-induced neuronal damage or loss by another mechanism.

Neurotransmitter receptors are single or multiple subunit macromolecules located in the neuronal plasma membrane. Binding of a specific neurotransmitter molecule to the corresponding site on the receptor initiates signal transduction in the neuron, resulting in excitation or inhibition. With ion-gated receptors, signal transduction involves the transient opening of an ion channel in the plasma membrane, accompanied by ion conductance. With other receptor types, excitation or inhibition is associated with biochemical changes leading to the generation of intracellular second messenger molecules.

Ethanol has been shown to have both acute and chronic effects on the function of several receptor systems. Some of these effects may underlie ethanol's acute and chronic effects on neuropsychological function and ethanol-induced brain damage. As we shall see, many of the neurotransmitter alterations after ethanol administration are reversible following a period of abstinence from ethanol, but some are not. We are suggesting that the irreversible nature of some of these changes may contribute to permanent impairment by setting in motion a series of intracellular events that leads to degeneration and ultimately death of neurons. In addition, the brains of ethanol-dependent animals may be particularly susceptible to receptor-mediated excitotoxicity

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during withdrawal from ethanol because of withdrawal-induced excitation of vulnerable neurons. Consequently, repeated episodes of chronic exposure, followed by withdrawal, may be more damaging than constant exposure to ethanol.

In the following discussion, we examine reports on animal studies of ethanol-induced changes in the properties and concentration of neurotransmitter receptors and attempt to relate these to current concepts of ethanol-induced brain damage. Where appropriate, the metabolism of transmitters and their effector second messenger systems is also considered.

GLUTAMATE RECEPTORS

Glutamate is the major excitatory neurotransmitter in the brain, which exerts its action through two families of receptors, ionotropic and metabotropic. The ion-gated receptors show selective sensitivity for N-methyl D-aspartate (NMDA), kainic acid, and α-amino-3-hydroxy-5-methyl-4-isoxazole propanoic acid (AMPA), respectively, and contain integral cation-specific ion channels (Foster and Fagg 1984; Monaghan et al. 1989; Watkins and Evans 1981). Of these, the NMDA receptor is the best characterized.

Recent studies on the interaction of ethanol and glutamate receptors suggest a major role, particularly for the NMDA receptor, in mediating ethanol-induced brain impairment and damage. The finding that ethanol, at concentrations as low as 5 mM, inhibits long-term potentiation in the hippocampus (Blitzer et al. 1990) provides an interesting possible explanation for ethanol-induced memory impairment and disruption of synaptic plasticity. However, major interest centers on increasing evidence that the sensitivity of NMDA receptors in the hippocampus is increased following chronic ethanol consumption and that this probably mediates seizures and excitotoxicity in the withdrawal state (see also Crews and Chandler, chapter 17).

The excitotoxicity hypothesis first proposed by Olney and coworkers (Olney et al. 1971) suggests that the neurotoxicity of glutamate and related compounds depends on excitation of neurons. In other words, the prolonged depolarization produced is itself cytotoxic. Initial suggestions implicated cellular swelling due to inward flow of sodium (Na^+) and chloride (Cl^-). More recently, attention has focused on the possibility that calcium (Ca^{2+}) entry may be the important factor.

It is now recognized that there are two components to excitotoxicity, differing in time-course and ionic dependence. There is an early component characterized by neuronal swelling and dependence on extracellular Na^+ and Cl^-. The late component features gradual neuronal disintegration and dependence on extracellular Ca^{2+}. Either component can produce irreversible neural damage, but the Ca^{2+}-dependent mechanism is probably more important, since damage can be shown at lower concentrations of glutamate (Choi 1987; Coyle et al. 1981). As a result, the damage may be mediated by intracellular Ca^{2+}-dependent, proteolytic enzymes, such as calpain.

The NMDA receptor is a receptor-ion channel complex with a variety of binding

sites for NMDA and regulatory agents, including glycine, magnesium (Mg^{2+}), zinc (Zn^{2+}), and polyamines. In addition, there is an intrachannel binding site mediating the action of noncompetitive antagonists, such as ketamine, phencyclidine, and dizocilpine (MK-801) (Foster and Fagg 1987). Activation of the NMDA receptor by glutamate is voltage dependent and appears to require occupation of the glycine and probably polyamine sites to overcome inhibition by Mg^{2+} and Zn^{2+}. Opening of NMDA channels results in a dramatic influx of Ca^{2+} as well as monovalent ions to mediate the postsynaptic effects (Yoneda and Ogita 1991). In contrast, metabotropic receptors function by intracellular signaling through guanine nucleotide-binding proteins (G-proteins).

Interactions between ethanol and the NMDA receptor have been investigated electrophysiologically and biochemically. It has been shown that ethanol, at low concentrations (5 and 50 mM), inhibits NMDA-activated currents in primary cultures of rat hippocampal and sensory neurons (Lovinger et al. 1989; White et al. 1990) and in hippocampal slices from adult rat brain (Lovinger et al. 1990). Blitzer et al. (1990) have shown that ethanol, at concentrations as low as 5 mM, inhibits long-term potentiation in rat hippocampal neurons, thus providing a biological correlate of ethanol-induced memory impairment. Concentrations of ethanol as low as 10 mM have been found to inhibit NMDA-stimulated Ca^{2+} uptake and generation of cGMP in primary cultures of rat cerebellar granule cells (Hoffman et al. 1989). In dissociated rat brain cells, ethanol inhibits the NMDA-stimulated increase in intracellular Ca^{2+} concentration (Dildy and Leslie 1989). Ethanol also inhibits [^{3}H]-noradrenaline release from rat brain cortex (Fink and Gothert 1990; Gonzales and Woodward 1990) and endogenous dopamine release from rat striatal slices (Woodward and Gonzales 1990) in response to NMDA stimulation. Explanations of ethanol's inhibitory actions include modulation by ethanol of the NMDA-glycine interaction at the NMDA receptor (Rabe and Tabakoff 1990; Woodward and Gonzales 1990) and interaction of ethanol with lipophilic regions of the NMDA binding site (Fink and Gothert 1990; Gonzales et al. 1991).

Available evidence suggests that chronic exposure of animals to ethanol leads to increased sensitivity of brain glutamate receptors, particularly the NMDA variety. This mechanism is thought to be important in producing an increased susceptibility to withdrawal seizures. An early report showed that chronic administration of ethanol to animals led to increased binding of L-glutamate to synaptic membranes (Michaelis et al. 1978). Ligand binding studies, using [^{3}H]-MK-801, have revealed an increased density of binding sites in the hippocampus of ethanol-fed mice. This was associated with an increased sensitivity to withdrawal seizures precipitated by NMDA administration and handling, whereas treatment of animals with MK-801 decreased the occurrence and severity of withdrawal seizures (Grant et al. 1990; Morrisett et al. 1990; Gulya et al. 1991).

Recent studies have indicated that, following intrahippocampal injection of NMDA, ethanol-dependent animals were more likely to die after seizures. There was a significant decrease in glutamate decarboxylase but not choline acetyltransferase activity in injected hippocampi of surviving animals, suggesting increased sensitivity of γ-aminobutyric acid (GABAergic) neurons to damage and death (Davidson et al. in press).

The hypothesis of glutamate-induced excitotoxicity is clearly the most attractive explanation for widespread receptor-mediated ethanol-induced brain damage. Moreover, it assists in rationalizing other findings, including hypofunction of GABA receptors and altered calcium homeostasis (see Crews and Chandler, chapter 17).

GABA RECEPTORS

Two distinct types of receptors for the major inhibitory amino acid GABA are found on vertebrate neurons. They are known as $GABA_A$ and $GABA_B$ receptors, and both varieties mediate neuronal inhibition. The $GABA_A$ receptor is composed of a GABA-benzodiazepine receptor-ionophore complex, which mediates neuronal inhibition through opening of an integral receptor-gated chloride channel. The resultant chloride influx results in hyperpolarization of the postsynaptic membrane. Action of the $GABA_B$ receptor, on the other hand, may result in the inhibition of opening of voltage-sensitive Ca^{2+} channels through mediation of a G-protein (Dunlap 1981; Simmonds 1983).

Most research has focused on the $GABA_A$ receptor. However, the few studies on the $GABA_B$ receptor have suggested a potential role for this receptor in mediating ethanol's action. Allan et al. (1991) showed that the $GABA_B$ agonist baclofen and the antagonist phaclofen modify ethanol-induced chloride flux in brain microsacs. However, these effects were not observed in cultured neurons (Mehta and Ticku 1990).

The $GABA_A$ receptor complex is functionally altered by ethanol exposure leading to hypofunction of this macromolecule in the tolerant state. The explanation for the loss of sensitivity to ethanol in tolerant animals is undoubtedly multifactorial and is related to altered function of the various ligand binding sites on the complex.

A reduction in expression of the α_1 and α_2 subunits of the $GABA_A$ receptor has recently been observed after chronic ethanol administration (Morrow et al. 1990; Montpied et al. 1991). These changes could lead to fewer "functionally available" GABA receptors, or functionally different $GABA_A$ isoreceptors, or a population of desensitized or nonfunctional receptors. It is interesting that similar changes have been observed after chronic ethanol treatment in mice genetically prone to withdrawal seizures (Buck et al. 1991*a*). Consequences of hypofunction of the $GABA_A$ receptor are discussed below.

The $GABA_A$ receptor complex exhibits three classes of binding sites, each binding a different type of ligand. First, there are low- and high-affinity sites for GABA (Enna and Snyder 1977; Ticku

1980*a*). Low-affinity $GABA_A$ receptors couple to chloride channels and are activated by GABA or muscimol at micromolar concentrations. Benzodiazepines are selectively bound by the second class, which is comprised of two subtypes. One of these, which is thought to mediate the sedative effects of benzodiazepines, is coupled to the chloride channel. The other, which is thought to mediate the anxiolytic effects of these drugs, is not connected to the chloride ionophore (Lippa et al. 1979). The third class of binding sites has been characterized with picrotoxinin, a convulsant that blocks GABA transmission without affecting GABA binding (Olsen et al. 1978). A variety of convulsant and depressant drugs, including barbiturates, also bind to this class of site (Ticku 1980*a*).

Studies on the molecular biology of the $GABA_A$ receptor have revealed at least five types of polypeptide subunits arranged to form four domains surrounding the transmembrane chloride channel. Many different subtypes have also been identified. Thus, there are at least six α, three β, and an undetermined number of γ, δ, and ε polypeptides. $GABA_A$ receptors from different brain areas have differing subunit composition. These differences in composition are probably responsible for regional differences in responses to drugs, including ethanol (Olsen and Tobin 1990).

Various electrophysiological studies have shown that ethanol augments the neural inhibitory effect of GABA (Davidoff 1975; Nestoros 1980*a*,*b*). This phenomenon could be mediated by one or more of the binding sites on the GABA-benzodiazepine receptor-ionophore complex. A recent investigation using antisense oligonucleotides indicates that ethanol sensitivity of the $GABA_A$ receptor is conferred by an eight-amino acid sequence contained in the γ2L subunit (Wafford et al. 1991).

GABA binding to rat and mouse brain preparations increases shortly after intraperitoneal injection of a single dose of ethanol (Ticku 1980*b*; Ticku and Burch 1980). This change is due to an increase in the density of low-affinity GABA receptors. On the other hand, ethanol has no effect on high-affinity binding of [^{3}H]-muscimol or [^{3}H]-GABA in vitro (Williams and Risley 1979; Marangos and Martino 1981; Greenberg et al. 1984).

Conflicting results have been found in chronic studies. GABA receptor density was decreased in mouse brain after 14 days on ethanol in drinking water (Ticku and Burch 1980) but not in rat brain after 21 days on an ethanol-containing liquid diet (Ticku 1980*b*). Following ethanol withdrawal, affinity of the low-affinity binding site was reduced, which correlated with increased sensitivity to audiogenic seizures (Ticku 1980*b*). Reggiani et al. (1980*a*) found an increase in density of high- and low-affinity GABA binding sites in brains of rats maintained on ethanol in drinking water for 21 days. A reduced density of muscimol binding sites was observed in rat brain after withdrawal from ethanol vapor inhalation (Linnoila et al. 1981). Withdrawn animals showed an increase in affinity for muscimol binding to cerebellar membranes (Nordberg et

al. 1985). Variations in the membrane preparations studied may explain these differences, especially considering the proposed role of membrane phospholipids in the function of GABA receptors and their sensitivity to detergents (Davis and Ticku 1981; Skerritt et al. 1982).

Interpretation of the effect of ethanol on benzodiazepine binding is also confounded by methodological problems. When assays are conducted at low temperature, ethanol (up to 2 M) does not alter binding characteristics of native or detergent-treated membranes (Freund 1980; Volicer and Biagioni 1982; Greenberg et al. 1984; Burov et al. 1985). However, at 25 to 37° C, ethanol (100 mM) reduces the affinity of benzodiazepine binding sites (Greenberg et al. 1984). By contrast, when membranes are solubilized with Lubrol-PX, ethanol enhances benzodiazepine binding, apparently through increasing receptor affinity (Davis and Ticku 1981; Ticku and Davis 1981; Ticku et al. 1984).

Chronic ethanol administration to animals generally does not affect binding of benzodiazepines to central type receptors (Liljequist and Engel 1984; Liljequist and Tabakoff 1985; de Vries et al. 1987; Hillmann et al. 1988). However, binding of the partial inverse agonist Ro15-4513 was significantly increased in rats chronically exposed to ethanol (Mhatre et al. 1988). Similar findings were obtained with cultured neurons grown in the presence of ethanol. These data suggest that enhanced binding capacity for inverse agonists may be one of the neural changes underlying tolerance and physical dependence (Mhatre and Ticku 1989). This conclusion is supported by the observation that administration of Ro15-1788, a benzodiazepine receptor antagonist, to ethanol-tolerant mice reduced signs of tolerance and dependence (Buck et al. 1991*b*). Changes have also been observed in the interrelationship of the GABA and benzodiazepine binding sites. Thus an increase in the EC_{50} and a lower level of maximal stimulation by GABA of flunitrazepam binding in mouse and rat brain cortical membranes was found after approximately 14 days of exposure to ethanol vapor (de Vries et al. 1987; Hillman et al. 1988). These findings may be interpreted as disruption of the interactions between polypeptide chains comprising the benzodiazepine receptor complex. Support for the concept is provided by the observation that chronic ethanol treatment reduces pentobarbital-induced stimulation of flunitrazepam binding in mice (Liljequist and Tabakoff 1985), and the suggestion that ethanol may uncouple sites in the GABA receptor and the chloride channel (Thyagarajan and Ticku 1985).

Interactions of ethanol with the third site on the GABA-benzodiazepine receptor-ionophore complex, the picrotoxinin binding site, have also been investigated. Several studies have shown potentiation by ethanol of GABA receptor-mediated chloride flux in vitro into synaptoneurosomes (Suzdak et al. 1986), microsacs (Allan and Harris 1986), and cultured spinal cord neurons (Ticku et al. 1986; Mehta and Ticku 1988). The intoxicant properties of ethanol have been attributed

to this effect, primarily because Ro15-4513, a selective antagonist of ethanol's anxiolytic and sedative effects, also antagonizes its stimulative action on GABA-mediated chloride flux (Nutt and Lister 1987; Suzdak et al. 1988). The difference in sensitivity to ethanol between long-sleep and short-sleep mice appears related to genetic differences in sensitivity of the $GABA_A$ receptor to stimulate chloride conductance (Allan and Harris 1986; Wafford et al. 1990).

There have been conflicting findings in some studies showing no interaction between ethanol and GABA (Morelli et al. 1988; Deitrich et al. 1989). This discrepancy may be due to methodological differences. For example, enhancement of GABA-stimulated $^{36}Cl^-$ uptake by ethanol in vitro is only observed in fresh tissue preparations (McQuilkin and Harris 1990). The possible involvement of $GABA_B$ receptors in mediating ethanol-induced changes in chloride flux is also controversial (Mehta and Ticku 1990; Allan et al. 1991).

The effect of ethanol on binding to the picrotoxinin site has been mainly examined using the ligand [^{35}S]-butylbicyclophosphorothionate (TBPS). Ethanol inhibits binding of TBPS to brain membranes in vitro (Thyagarajan and Ticku 1985; Liljequist et al. 1986). Acute dosage of animals with ethanol produces a similar effect (Thyagarajan and Ticku 1985; Sanna et al. 1991). Several studies of chronic administration of ethanol showed no effect on the affinity or density of TBPS binding sites in brain (Liljequist et al. 1986; Rastogi et al. 1986). However, a recent study comparing three methods of ethanol administration reported decreased affinity and increased density of TBPS binding sites in brains of rats maintained on ethanol in drinking water for 3 months (Hillmann et al. 1990). Other methods, including a liquid diet and ethanol vapor inhalation for shorter periods, were without effect. These findings with TBPS binding are consistent with the report that chronic ethanol administration, leading to tolerance and dependence, is associated with abolition of ethanol-mediated potentiation of muscimol-stimulated $^{36}Cl^-$ uptake by synaptoneurosomes (Morrow et al. 1988). This change was reversible following ethanol withdrawal.

Thus, most studies of the chronic effects of ethanol point to hypofunction of $GABA_A$ receptors. Since GABA is the major inhibitory neurotransmitter in the brain, hypofunction of the $GABA_A$ receptor complex has important implications for neuronal excitability. It implies that neurons (e.g., glutamatergic neurons) normally subject to inhibition via $GABA_A$ receptors will be disinhibited, contributing to the increased neural excitability observed in dependent animals following ethanol withdrawal. Such disinhibited neurons would therefore be predisposed to damage by the mechanisms underlying excitotoxicity.

ACETYLCHOLINE RECEPTORS

Acetylcholine receptors in the central nervous system are classified pharmacologically into two major divisions, nicotinic and muscarinic. Central muscarinic receptors are thought to be involved in the

processes of learning and memory (Davis et al. 1976; Bartus et al. 1982; Dunne and Hartley 1985). Alterations in the function of these receptors have been implicated in various types of dementia, including ethanol-induced brain damage (Arendt et al. 1983; see also Arendt, chapter 22).

The changes in muscarinic acetylcholine receptors following chronic ethanol exposure are probably secondary to loss of neurons in the basal forebrain cholinergic projection system with a compensatory increase in postsynaptic muscarinic binding sites. Arendt et al. (1988) found a reduction in the number of acetylcholinesterase-positive neurons in the basal nucleus of Meynert in rats after 12 weeks on ethanol-containing drinking water (20 percent v/v). This change was associated with a decrease in the activity of the cholinergic marker enzymes choline acetyltransferase and acetylcholinesterase in the basal forebrain. They attributed these findings to a neurotoxic effect of ethanol on cholinergic neurons, leading to partial cholinergic denervation of the cerebral cortex, hippocampus, and amygdala. No information is presently available on the mechanisms underlying the proposed neurotoxicity, nor is there any indication why cholinergic neurons of the basal forebrain projection system should be particularly vulnerable to ethanol or its metabolites. It is interesting that the memory deficit and measures of cholinergic activity improve after transplants of cholinergic-rich fetal basal forebrain cell suspensions into cortex, hippocampus, or both these brain areas (Arendt et al. 1989; Arendt, chapter 22).

Earlier studies with the classical antagonist quinuclidinyl benzilate (QNB) suggested a homologous population of binding sites on brain muscarinic receptors. Subsequently, it has been shown that selective agonists and antagonists can distinguish binding sites with low-, high-, and super high-affinity that are interconvertible by the presence of ions or guanyl nucleotides (Birdsall et al. 1978; Hulme et al. 1983).

Using the novel antagonist pirenzepine, Hammer et al. (1980) distinguished two subclasses of muscarinic receptors, M_1 and M_2. M_1 receptors are thought to be excitatory in nature, whereas M_2 receptors are inhibitory (Potter et al. 1984). Autoradiographic studies show different localizations of M_1 and M_2 receptors in the brain. Seventy to ninety percent of muscarinic receptors in the cerebral cortex, hippocampus, and striatum are M_1 receptors, whereas those in the pons and medulla oblongata are predominantly M_2 (Potter et al. 1984; Messer and Hoss 1987). M_1 receptors are located postsynaptically mainly on dendritic tufts (Vogt and Burns 1988), and M_2 are located presynaptically on axon terminals (Marchi and Raiteri 1985; Vogt and Burns 1988).

Gil and Wolfe (1985) have proposed that M_1 and M_2 receptors are linked to different effectors, M_1 being coupled to phosphoinositol (PI) breakdown and M_2 to inhibition of adenylyl cyclase activity. Muscarinic receptor heterogeneity was further confirmed by the discovery of four separate genes coding for muscarinic receptor proteins m1, m2, m3, and m4 (Kubo et al. 1986, 1987; Bonner et al. 1987; Peralta et al. 1987). At least three of

these subtypes (m1, m3, and m4) are present in rat brain and show distinct regional distributions (Brann et al. 1988). Subtypes m1 and m3 stimulate PI hydrolysis but do not inhibit adenylyl cyclase, whereas m2 and m4 inhibit adenylyl cyclase (Peralta et al. 1988).

Ethanol (up to 500 mM) does not affect the binding of ligands to brain muscarinic receptors in vitro (Pietrzak et al. 1988). Similarly, acute treatment of animals with ethanol has no effect on the binding of ligands to these receptors (Wigell and Overstreet 1984).

In contrast, chronic treatment with ethanol usually leads to regionally selective increases in muscarinic receptor density. However, the results of animal studies are greatly influenced by the strain of animal, method of ethanol administration, and duration of treatment. In mice fed an ethanol-containing liquid diet for 7 to 8 days, receptor density increases in the cerebral cortex and hippocampus but not striatum (Tabakoff et al. 1979; Rabin et al. 1980; Smith 1983). In rats the time required to produce these changes is much longer, and the method of administration seems crucial. An increased density of cortical receptors following ethanol inhalation for 14 days was only detectable in rats younger than 6 weeks (Dunglison et al. 1989). No changes were found in cortical, hippocampal, or striatal receptors following daily injections for 1 to 2 months (Wigell and Overstreet 1984). Gastric intubation for 11 to 15 days resulted in small changes (5 to 7 percent), which are probably physiologically insignificant (Muller et al. 1980).

Following longer periods of ethanol administration, marked changes in muscarinic receptor density are revealed. Pietrzak et al. (1988) found a significant increase (50 percent) in cerebral cortical receptors in male Wistar rats after 3 months on ethanol (15 percent v/v) as the only source of fluid. Female, but not male, Long-Evans hooded rats showed an increased density of cortical, hippocampal, and striatal receptors after 4 months on an ethanol-containing liquid diet (Witt et al. 1986). After treatment of male Wistar rats with ethanol-containing water at restricted times for about 15 months, increased density of cortical and striatal receptors was demonstrable in rats undergoing withdrawal (Nordberg and Wahlstrom 1982; Nordberg et al. 1985).

The use of selective ligands to differentiate receptor subtypes reveals the effects of ethanol to be regionally selective. Pietrzak et al. (1989) assayed muscarinic receptors in rats treated continuously with ethanol for up to 2 years. At 3 and 9 months, there was an increased density of cortical muscarinic receptors. However, after further treatment (15, 21, and 25 months) this effect was no longer evident. In the striatum there was no significant change in the density of muscarinic receptors until 15 months of treatment. Similarly, in the hippocampus 21 months of exposure were required to produce significant changes. In general, the increase in the total number of cortical muscarinic receptors can be explained by an increase in the density of M_1-binding sites existing in the low-affinity agonist state (Pietrzak et al. 1989).

The increase in M_1 sites, which are located postsynaptically, may be a reflection of dendritic sprouting known to occur following long-term exposure to ethanol (Arendt, chapter 22). Another factor could be the impairment of G-protein function observed with chronic ethanol ingestion (see below). Thus, cholinergic transmission may be impaired by losing cholinergic neurons and interfering with the efficacy of transmission at the remaining functional cholinergic synapses. At this stage, it is not clear how these changes are related to the more severe disturbances of cholinergic function involving neuronal loss. Possibly, the cholinergic neurons of the basal forebrain are particularly susceptible to ethanol-induced damage unrelated to receptor changes, such as that resulting from free radical reactions (see Hunt, chapter 15; Pellmar, chapter 16).

DOPAMINE RECEPTORS

Dopamine receptors have been extensively characterized and subdivided into two subtypes, D_1 and D_2, differentiated by biochemical, physiological, and pharmacological features. D_1 receptors stimulate adenylyl cyclase, resulting in an increase in cyclic AMP, and have low affinity for dopaminergic antagonists, such as spiroperidol and haloperidol. D_2 receptors have a higher affinity for these antagonists but a lower affinity for dopamine. Occupation of D_2 receptors is associated with inhibition of adenylyl cyclase activity.

Alterations in the function of dopaminergic receptors are associated with chronic ethanol administration. From this discussion, tolerance and physical dependence apparently involve hypofunction of these receptors, but may be later replaced by hyperfunction during the withdrawal state. This latter effect may contribute to the hyperexcitability seen during withdrawal.

Several studies have attempted to find the role of alterations in dopamine receptor activity in mediating the biological effects of ethanol. Using mice treated with ethanol in a liquid diet for 7 days, Tabakoff and colleagues monitored several biochemical and behavioral parameters related to dopaminergic drug action. Both dopaminergic agonists and antagonists were less effective 24 hours after withdrawal (Hoffman and Tabakoff 1977; Tabakoff et al. 1978; Tabakoff and Hoffman 1978; Black et al. 1980). These results suggest a loss of sensitivity of the dopaminergic system or hypofunction in the intoxicated animal. This idea has been supported by the work of Leslie et al. (1986), who have shown uncoupling between Ca^{2+} influx and dopamine release in dependent animals. This uncoupling can be overcome by addition of ethanol in vitro to synaptosomal preparations. Similarly, Lynch et al. (1985) have indirectly shown a hypofunctioning dopaminergic system in striatal sections from dependent animals.

Several observations implicate dopaminergic hypofunction during withdrawal in the short-term, ethanol-dependent animal, including reduced dopamine turnover (Hunt and Majchrowicz 1974), reduced dopamine release (Darden and Hunt 1977), and reduction in withdrawal

symptoms following intraventricular administration of haloperidol (Blum et al. 1976). The initial phase of hypofunction transforms into a period of hyperfunction after longer-term ethanol exposure (Liljequist and Engel 1979). Duration of ethanol administration may explain some inconsistencies found concerning the reported changes in sensitivity (hyper versus hypo) of dopamine receptors during withdrawal (Engel and Liljequist 1976; Hoffman and Tabakoff 1977; Tabakoff et al. 1978; Tabakoff and Hoffman 1979).

Recent molecular cloning studies have revealed that the pharmacologically defined receptors are members of a larger family of dopamine receptors. To date an additional three receptors (D_3, D_4, and D_5) have been described in human brain. All these receptors belong to a large superfamily of receptors that have seven transmembrane domains and are coupled to intracellular transduction systems by G-proteins (Schwartz et al. 1992).

The D_2 receptor has been shown to exist in two protein isoforms differing in length because of alternative mRNA slicing (Monsma et al. 1989). This suggests functional differences, although both forms seem to have identical actions and pharmacological profiles. The distribution of these receptors correlates well with dopaminergic projections; areas with the highest levels are found in the caudate-putamen, nucleus accumbens, and olfactory tubercule. When expressed in mammalian cells, the cloned rat D_2 receptor exhibits specific ligand binding activity and functional coupling to inhibition of adenylyl cyclase activity (Bunzow et al. 1988). A closely related third type of receptor (with 52 percent overall homology) has recently been described and termed D_3 (Sokoloff et al. 1990). When expressed in mammalian cells, the D_3 receptor exhibits similar pharmacological properties to those of the D_2 receptor, but is distinct in that it does not display inhibition of adenylyl cyclase activity, which is characteristic of the D_2 receptor.

Marked changes in many aspects of dopamine metabolism and release occur after ethanol treatment. Low doses of ethanol stimulate dopamine release (Darden and Hunt 1977; Holman and Snape 1985; Lynch et al. 1985), dopamine synthesis (Carlsson and Lindquist 1973), and dopamine turnover (Carlsson et al. 1973), and increase the cerebral concentration of dopamine metabolites (Karoum et al. 1976). At higher doses, ethanol decreases dopamine release (Darden and Hunt 1977), reduces uptake (Bacopoulos et al. 1978), and stimulates dopamine synthesis (Carlsson and Lindquist 1973). These biphasic changes are confirmed by behavioral and electrophysiological studies (Carlsson et al. 1972; Mereu et al. 1984). The effect of ethanol may be due to a direct action on dopaminergic neurons (Carlsson et al. 1972) or may be indirect, either via removal of the inhibitory action of GABAergic pathways (Mereu and Gessa 1985; Samuel et al. 1983) or by release of an opioid peptide that stimulates opioid receptors on the presynaptic endings of dopaminergic neurons, thereby facilitating the release of transmitter (Widdowson and Holman 1986).

Ethanol at concentrations below 50 to 100 mM in vitro has no effect on [^{3}H]-spiroperidol binding to dopamine receptors in the caudate nucleus (Hruska and Silbergeld 1980; Reggiani et al. 1980*b*). At higher concentrations ethanol dose-dependently reduces [^{3}H]-spiroperidol binding, which can be explained by a reduction in the affinity of the receptors, rather than a loss of binding sites (Hruska and Silbergeld 1980). In contrast, Rabin and Molinoff (1981) were unable to find any effect below an ethanol concentration of 750 mM, and Hunt and Dalton (1981) found no effect on the binding of several different ligands.

An acute oral dose of ethanol (3 g/kg) fails to produce any effect on the binding of [^{3}H]-spiroperidol (Barbaccia et al. 1980; Lai et al. 1980). However, Silverman (1987) reported that, in mice with a unilateral lesion of the nigrostriatal pathway, intraperitoneal injection of ethanol induces dose-dependent apomorphine-like rotation, which suggests direct activation of dopaminergic receptors.

Results of studies of the effect of chronic ethanol treatment differ depending on the method and duration of treatment. After 4 to 7 days of treatment, the binding characteristics of dopamine receptors in the caudate nucleus are unchanged (Hunt and Dalton 1981; Rabin et al. 1980; Tabakoff and Hoffman 1979). After 14 days of treatment with daily doses of 6 g/kg, binding to dopaminergic receptors is elevated (Lai et al. 1980). Similarly, administration of ethanol in the drinking water increases D_2 receptor density in rat striatum (Fuchs et al. 1987). Hruska (1988) emphasized the importance of the duration of ethanol treatment and showed that after 21 days on a liquid diet, but not 14 days, there is an increase in density of both D_1 and D_2 receptors in the striatum. In a recent study, Hamdi and Prasad (1992) recorded a biphasic response of striatal D_2 receptors, with an initial reduction in density followed by an increase to levels above the controls. Long-term treatment of 32 weeks results in a reduced density of receptors in the striatum (Syvalathti et al. 1988). A similar response is seen following shorter treatment (13 weeks) but only after 4 weeks of withdrawal. Therefore, dopaminergic function appears impaired by chronic exposure to ethanol. This outcome is probably a combination of presynaptic and postsynaptic effects culminating, at first, in hyposensitivity of dopamine receptors, followed by hypersensitivity in the withdrawal state. This hypersensitivity could play a role in predisposing to excitotoxic damage during withdrawal.

Recently, however, Hietala et al. (1990) have claimed that the changes in number and affinity of cortical and striatal D_1 receptors and striatal D_2 receptors in rat brain are due to dietary factors associated with the method of ethanol administration, rather than ethanol per se. Further work is required to clarify this issue.

NOREPINEPHRINE RECEPTORS

A variety of adrenergic receptors have now been identified. These subdivide into two major groups, α- and β-receptors. Originally based on ligand binding data, the differentiation has been confirmed by molecular cloning. To date, three groups

of β-receptors have been found and their protein sequences determined. β-adrenergic receptors stimulate adenylyl cyclase. The two classes of α-adrenergic receptors, α_1 and α_2, were originally distinguished by differential sensitivity to alkylation and subsequently identified as the vascular smooth muscle receptor (α_1) and the presynaptic inhibitory receptor of norepinephrine release (α_2). Six different α-adrenergic receptors have been identified by molecular cloning. It would appear that, in the mammalian genome, there may be two sets of three genes coding for the six α-receptors.

A body of evidence from behavioral studies implicates adrenergic systems in the physiological and behavioral effects of ethanol. Clearly, depression of central adrenergic activity increases ethanol consumption in animals, despite the method used for induction of depression (Mason et al. 1979; Aalto and Kiianmaa 1984, 1987; Hilakivi et al. 1987). Conversely, stimulation of central noradrenergic activity reduces voluntary ethanol consumption by rodents (Daost et al. 1987; Guaza et al. 1986; Tabakoff and Ritzmann 1977; Melchior and Tabakoff 1981). The acute and chronic effects of ethanol on adrenergic receptor function concerning acute intoxication and the development of tolerance, physical dependence, and brain damage have also been investigated.

Low concentrations of ethanol reversibly alter the affinity of the high-affinity form of the β-adrenergic receptors in mouse cerebral membranes in vitro (Hoffman et al. 1987). A direct effect on the receptor or its G-protein has been suggested to account for this result.

After 4 days of ethanol treatment there was no effect on the characteristics of the α- or β-receptors in brain (Hunt and Dalton 1981). By increasing the time of exposure to 7 to 60 days the apparent density of β-receptors was reduced without effect on α-receptors (Banerjee et al. 1978; Muller et al. 1980; Rabin et al. 1980). After 60 days of exposure, β-receptor binding was measured during intoxication and 3 days after withdrawal (Banerjee et al. 1978). Binding was reduced during intoxication but elevated during the withdrawal period. A similar elevation during withdrawal was reported by Kuriyama et al. (1981). Other studies on the chronic effect of ethanol on α-adrenergic receptors have shown few changes in receptor characteristics, either in binding (Ciofalo 1979) or in terms of sensitivity, as measured by the effects of clonidine (Liljequist et al. 1978). The implications of the findings from these various studies are similar to those discussed under dopaminergic receptors.

SECOND MESSENGER SYSTEMS

The actions of many neurotransmitters are mediated by second messengers. The extent to which effects of ethanol on second messenger systems may mediate changes observed in the function of receptors and consequently ethanol-induced impairment has been the subject of considerable investigation (see below).

The variety of signal transduction mechanisms demonstrated in the brain has increased markedly in recent years. Common elements in many receptor-

effector interactions are the G-proteins. These are heterodimers consisting of α-, β-, and γ-subunits. The α-subunit contains the receptor and effector binding domains, the guanosine triphosphate (GTP) binding site, and GTPase activity. Many of the genes coding for the G-proteins have now been cloned.

Analysis of these genes has suggested that variations in the α-subunit confer specificity for interaction with receptors and effectors. Also, the likely number of G-proteins present in the tissues is very large. The control of adenylyl cyclase activity by several transmitters, including dopamine, serotonin, norepinephrine, and histamine (Nathanson 1977), is via G_s (stimulatory), whereas inhibition by receptors binding morphine, enkephalin, and acetylcholine in the striatum is via G_i (inhibitory).

Apparently, the effects of chronic ethanol administration on a number of receptor systems linked to adenylyl cyclase may be due to alterations in the expression of the transducer protein, $G_{\alpha s}$ (Mochly-Rosen et al. 1988). Similar findings have been obtained in studies on receptors linked through G-proteins to hydrolysis of PI (Pietrzak et al. 1990). It is tempting to speculate that the primary action of ethanol on these receptor systems may be on the G-proteins or their coupling to receptors. Also, this may be another unitary theme explaining the effect of ethanol on certain neural receptors and their function.

The effect of ethanol on adenylyl cyclase in vitro is marked. Ethanol increases adenylyl cyclase activity reversibly in membranes isolated from the striatum, cerebral cortex, hippocampus, and cerebellum (Rabin and Molinoff 1981; von Hungen and Baxter 1982; Saito et al. 1985) and in membranes isolated from lymphoma cells (Bode and Molinoff 1988). The ethanol effect is present in S49 cell lines that possess a G-protein but not in those lacking it (Rabin and Molinoff 1983). Luthin and Tabakoff (1984) found that inclusion of GTP or guanosine-5'-[β,γ-imido]triphosphate [Gpp(NH)p], a nonhydrolyzed analogue of GTP, in the assay reduced the concentration of ethanol required to produce its effect. Furthermore, Bode and Molinoff (1988) found that ethanol shifted the dose-response curve for a number of agonists. Neither maximum reaction velocity nor affinity of the solubilized enzyme is affected by ethanol (Hoffman et al. 1987; Rabin and Molinoff 1983). Together these data suggest an effect of ethanol in vitro on the receptor coupling rather than on adenylyl cyclase itself.

Cortical adenylyl cyclase activity, which is stimulated by β-adrenergic agonists, is also stimulated by ethanol. The EC_{50} for magnesium activation and the lag-time for Gpp(NH)p stimulation is reduced by ethanol (Saito et al. 1985). One can conclude that ethanol promotes the dissociation of the G_s protein from the enzyme. Ethanol does not appear to alter the inhibition of striatal adenylyl cyclase activity produced by morphine, enkephalin, and acetylcholine, an effect mediated through the G_i protein (Rabin 1985; Hoffman and Tabakoff 1986).

Early work on the effects of chronic ethanol administration (Israel et al. 1972,

French et al. 1975) showed decreased stimulation of cortical adenylyl cyclase activity by norepinephrine in preparations from treated animals. More recently, the effect of chronic ethanol treatment on cortical membranes has been studied in greater detail (Saito et al. 1987). These authors concluded that the changes observed are not due to alterations in the catalytic subunit but to a decreased amount of G_s. Examination of the affinity of the β-adrenergic receptor also indicates that the cortical receptor is uncoupled after chronic ethanol treatment, reminiscent of homologous desensitization (Hoffman et al. 1987). Although direct measurements by Western blots and specific antibodies did not show any changes in $G_{\alpha s}$ after chronic ethanol ingestion, Valverius et al. (1988) and Nhamburo et al. (1988) showed reduced concentration of $G_{\alpha s}$ protein in selected brain areas, using the ribosylation properties of cholera toxin. Similar experiments to assay the effects of ethanol on G_i by Western blotting and by pertussis toxin failed to detect any change. Results of studies with brain support the postulate that chronic ethanol ingestion produces a region-specific alteration in the properties and function of $G_{\alpha s}$ while having little or no effect on $G_{\alpha i}$.

The G_s protein as a target for ethanol action has been confirmed in cell culture. In NG108-15 (neuroblastoma-glioma hybrid) cells, ethanol-induced changes in adenylyl cyclase activity could be related to a decrease in quantity of $G_{\alpha s}$ mRNA. There was no change in the quantity of G_i mRNA. However, in other neural cell lines, the mechanism underlying ethanol-induced changes in the response of adenylyl cyclase to agonists varied. In N1E-115 cells treated with ethanol at high concentrations for 48 hours, a large increase in $G_{\alpha i}$ was found. After further incubation an increase in $G_{\alpha s}$ was also observed (Mochly-Rosen et al. 1988). Other cells, such as N18TG2 and one clone of PC12 cells, did not show any marked response to chronic ethanol treatment (Charness et al. 1988; Rabe et al. 1990). The explanation of these discrepancies remains to be investigated.

Another second messenger system that has been studied in relation to ethanol's action is agonist-induced breakdown of PI. Acute treatment with ethanol does not affect receptor-stimulated PI metabolism (Smith et al. 1986). However, there appears to be general agreement that the EC_{50} for the agonist carbachol is reduced after chronic ethanol treatment (Hoffman et al. 1986; Smith et al. 1986: Pietrzak et al. 1990), probably reflecting the upregulation of M_1 receptors (Hoffman et al. 1986; Smith et al. 1986; Pietrzak et al. 1988, 1989). An additional effect in the form of reduced maximal stimulation is noted after treatment with ethanol in drinking water for 3 months (Pietrzak et al. 1990). The latter effect is large enough to overcome the increased sensitivity resulting from the decreased EC_{50}, such that at any agonist concentration the response is markedly attenuated. This response suggests that ethanol exposure may result in an uncoupling of the receptor from the effector, but that upregulation of the receptor may be a

secondary, compensatory change resulting from this uncoupling.

A third example of a second messenger system, which has been extensively studied in relation to the effects of ethanol, is the calcium ion. Ca^{2+} is a ubiquitous link in stimulus-secretion coupling. It has long been recognized that ethanol can inhibit stimulated release of a range of transmitters from nerve terminals (Carmichael and Israel 1975), through inhibition of Ca^{2+} uptake via voltage-dependent calcium channels (VDCCs) (Harris and Hood 1980; Leslie et al. 1983).

Acute and chronic administration of ethanol has been shown by several workers to lead to a proliferation of VDCC in neuronal membranes, as demonstrated by the binding of dihydropyridine antagonists, such as nimodipine and nitrendipine (Rius et al. 1987; Dolin et al. 1987). Similar findings have been obtained with cultured cells (Brennan et al. 1989). This phenomenon may underlie hyperexcitability in dependent animals following withdrawal. Support for this concept comes from observations that administration of dihydropyridines can delay the formation of tolerance to ethanol (Wu et al. 1987; Pucilowski et al. 1989) and prevents seizures in dependent animals undergoing withdrawal (Little et al. 1986).

Since the effective intracellular free Ca^{2+} concentration, $[Ca^{2+}]_i$, is the ultimate mediator of excitotoxic neural damage, the density of VDCC in neuronal membranes is likely an important factor determining the fate of cells exhibiting increased sensitivity of glutamate receptors and hypofunction of GABA receptors. In terms of this hypothesis, chronic ethanol administration produces neuronal hyperexcitability through upregulation of glutamate receptors and downregulation of the counterbalancing GABA receptors. The hyperexcitable cells are much more susceptible than normal cells to excitatory stimuli. Combined with the increased density of VDCCs in the neuronal membrane, the change in susceptibility promotes the development of abnormally high $[Ca^{2+}]_i$ that triggers cell damage through activation of Ca^{2+}-dependent enzymes, such as neutral proteases.

Thus, a number of the effects of chronic ethanol exposure, which could be expected to impair brain function, may be due to alterations in receptor-effector coupling. In some cases, this involves decreased function, for example, through decreased expression of a stimulatory G-protein. In other cases there may be enhancement of function, such as in Ca^{2+}-dependent systems where the density of VDCCs in the neuronal membrane is increased. However, this enhancement sensitizes the neuron to subsequent Ca^{2+}-mediated damage.

SUMMARY AND CONCLUSIONS

Neurotransmitter receptor systems may be involved both directly and indirectly in causing changes in brain function. Direct involvement implies that ethanol-induced alterations in receptor function lead, first, to impaired neuropsychological function, which is reversible on withdrawal of ethanol. At more advanced stages, the receptor changes mediate a state of hyperexcitability that may predispose neurons

to degeneration and death. Events precipitating these irreversible changes are likely to be those associated with ethanol withdrawal in dependent animals. Specifically, hypersensitivity of glutamate (particularly NMDA) receptors, accompanied by hypofunction of GABA receptors on the same neurons, may lead to excitotoxic damage and cell death, following seizures induced by ethanol withdrawal. The fact that $[Ca^{2+}]_i$ is an important factor in activation of processes responsible for neuronal damage suggests that an increase in VDCCs, which also occurs after chronic ethanol exposure, contributes significantly to excitotoxicity.

Alterations in dopaminergic and muscarinic cholinergic receptors after chronic ethanol exposure may be the result of ethanol-induced impairment of receptor-effector coupling. Alternatively, with muscarinic receptors the changes may be a reflection of dendritic sprouting and other processes in response to ethanol-induced neurotoxicity that is not mediated via receptors.

There is clearly considerable scope for further research on receptor changes associated with ethanol-induced brain damage. A powerful stimulus is the rapidly increasing knowledge of the molecular biology and function of neurotransmitter receptors. As understanding of the mechanisms underlying receptor-mediated neurotoxicity increases, so will the prospect of intervention designed to prevent irreversible brain damage.

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POSSIBLE MOLECULAR MECHANISMS OF DAMAGE AND THERAPEUTIC STRATEGIES TO REDUCE DAMAGE OR IMPROVE FUNCTION

15

ROLE OF FREE RADICAL REACTIONS IN ETHANOL-INDUCED BRAIN DAMAGE: AN INTRODUCTION

Walter A. Hunt, Ph.D.[1]

INTRODUCTION

The chapters in this section consider several possible mechanisms by which chronic ethanol consumption can lead to brain damage and behavioral dysfunction. One suggested mechanism is that free radicals play a role in apparently permanent alterations in neuronal structure and function after long-term ethanol consumption, leading to cell death (Pellmar, chapter 16; Crews and Chandler, chapter 17; Lancaster, chapter 18). Free radicals are molecules containing unpaired electrons and are usually very reactive species, providing the potential for damage to biological tissue. However, most are quite unstable and short-lived, having lifetimes sometimes as short as 0.1 nanosecond. Consequently, free radicals that induce damage must be produced close to the site of action.

Under normal physiological conditions, a balance exists between the free radicals that are oxidants (prooxidants) and the scavengers of free radicals (antioxidants). However, conditions can arise in which this balance is altered, whereby the ratio of prooxidants to antioxidants increases. The resulting oxidative stress can lead to a greater likelihood of tissue damage (Sies and Cadenas 1985).

Oxidative stress and free radical damage have been implicated in several neurological diseases. A notable case is stroke (Floyd 1990) in which injury to the brain results from transient ischemia. Initially, tissue is deprived of oxygen for a period, then is reoxygenated. Damage occurs during this latter period. During ischemia, less adenosine triphosphate (ATP) is produced, and its precursor adenosine monophosphate is converted to adenosine, then to xanthine. When oxygen is restored in the tissue, the xanthine is converted by xanthine oxidase to uric acid and superoxide radical. The superoxide radical can then be converted to other radicals by reactions 2, 4, and 5, and form lipid peroxides, as described later.

Free radicals may also underlie other neurological disorders. These include epilepsy (Pazdernik et al. 1992), compli-

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cations of ozone exposure (Rahman et al. 1992), schizophrenia, tardive dyskinesia, and Parkinson's and Alzheimer's diseases (Jesberger and Richardson 1991). Normal aging may also involve excessive actions by free radicals (Jesberger and Richardson 1991; LeBel and Bondy 1992).

This chapter provides a brief overview of free radicals in the brain and their possible role in the actions of ethanol, including how free radicals form and the consequences of their presence on neuronal function. Also, mechanisms by which ethanol exposure can enhance the formation of free radicals and subsequent reactions involving free radicals will be explored. Finally, the possibility of using free radical scavengers as a possible therapeutic strategy to treat ethanol-induced brain damage will be noted.

BASICS OF FREE RADICAL REACTIONS

All aerobic cells, including those in the brain, require oxygen to survive. Two oxygen atoms are normally combined as an oxygen molecule that is itself a relatively stable, free radical. However, the metabolism of oxygen can form other free radicals, with the potential for toxic consequences on cell constituents (see reviews by Halliwell and Gutteridge 1985, 1989; Floyd 1990). Generally, cells defend against oxygen-induced damage using protective enzymes and antioxidants. However, when abnormally high amounts of free radicals are formed or when the protective mechanisms are impaired, cellular damage can result.

When oxygen is metabolized, hydrogen peroxide can be formed by a two-stage addition of electrons and protons involving the superoxide radical as an intermediate.

$$O_2 + e^- = = \Rightarrow O_2^- \quad (1)$$

$$O_2^- + e^- + 2H^+ = = \Rightarrow H_2O_2 \quad (2)$$

Some metals, called transition metals, can enhance the formation of free radicals, by accepting or donating a single electron. For example, iron can be either Fe(II) or Fe(III) ions, differing by one electron. The Fe(II) ion can be easily oxidized to the more stable Fe(III) ion by donating one electron. One way to do this is reduction of oxygen, resulting in the formation of the superoxide free radical.

$$Fe^{2+} + O_2 = = \Rightarrow Fe^{3+} + O_2^- \quad (3)$$

In addition, Fe(II) can also be oxidized by hydrogen peroxide to form the hydroxyl radical and the hydroxide ion through the Fenton reaction.[2]

$$Fe^{2+} + H_2O_2 = = \Rightarrow$$
$$Fe^{3+} + \cdot OH + OH^- \quad (4)$$

Copper, whose concentration is highest in the brain and liver, can transfer single electrons, as does Cu(I) with hydrogen peroxide to form hydroxyl radicals and hydroxide ions.

$$Cu^+ + H_2O_2 = = \Rightarrow$$
$$Cu^{2+} + \cdot OH + OH^- \quad (5)$$

[2] *The dot in reaction 4 and in subsequent reactions represents an unpaired electron.*

The hydroxyl free radical is one of the most reactive radicals known and can oxidize nearly any other molecule. Oxidative attack by the hydroxyl free radical is especially effective against lipids containing polyunsaturated fatty acids because of their numerous reactive hydrocarbon bonds (Halliwell and Gutteridge 1989).

LIPID PEROXIDATION OF MEMBRANES

Lipid peroxidation is a process that readily modifies polyunsaturated lipids, a particularly important phenomenon in biological membranes, where it can have profound effects on the normal functioning of a cell. Biological membranes are generally believed to consist of a bimolecular leaflet (figure 1). They are composed of two layers of phospholipids, with their polar head groups positioned on the outside and inside surfaces of the membrane and with their nonpolar hydrocarbon chains positioned end-to-end in the interior of the membrane. The hydrocarbon chains are saturated or unsaturated and generally lie in a plane perpendicular to the surface of the membrane. Biological membranes also contain proteins that confer specific biological properties to the membrane. An appropriate lipid environment is important in optimizing these membrane properties and the oxidation of polyunsaturated fatty acids of phospholipids in biological membranes.

Lipid peroxidation in the brain as in other tissues begins with the abstraction of a hydrogen atom from a methylene

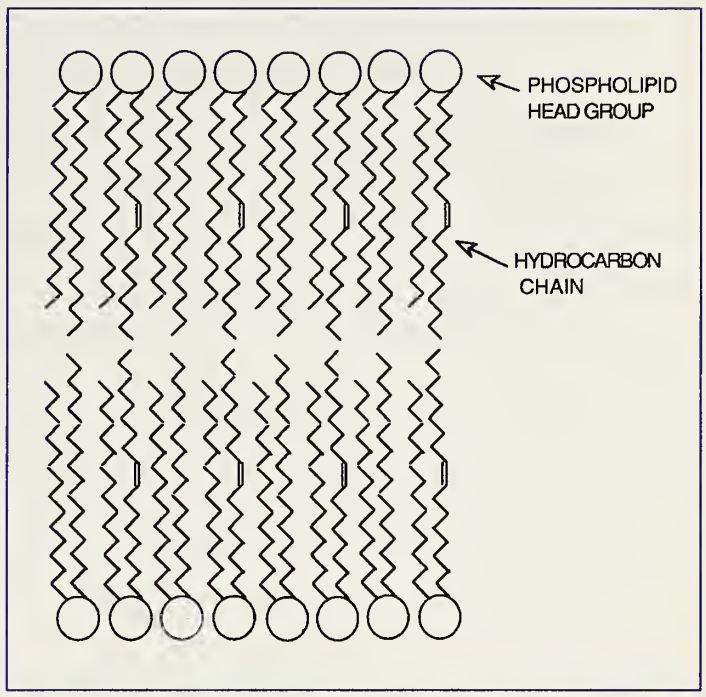

FIGURE 1

Schematic representation of the lipid bilayer of a biological membrane.

group, especially if it is adjacent to a double bond[3] (figure 2). This initial free radical molecule then rearranges into a conjugated diene that can react with an oxygen molecule to form a peroxyl radical (ROO·). Peroxyl radicals can abstract hydrogen atoms from other lipid molecules to form lipid hydroperoxides (ROOH) and new peroxyl radicals. These reactive radicals then continue to abstract other hydrogen atoms, setting in motion a chain reaction that can oxidize a significant amount of the unsaturated fatty acids in a membrane before the chain is terminated. Lipid peroxidation ends when no further reactive free radicals are produced.

The intact lipid hydroperoxides (ROOH) are relatively stable but can readily react with the transition metals, such as iron and copper, to form either

[3] *For a complete discussion of lipid peroxidation, see Halliwell and Gutteridge (1985, 1989).*

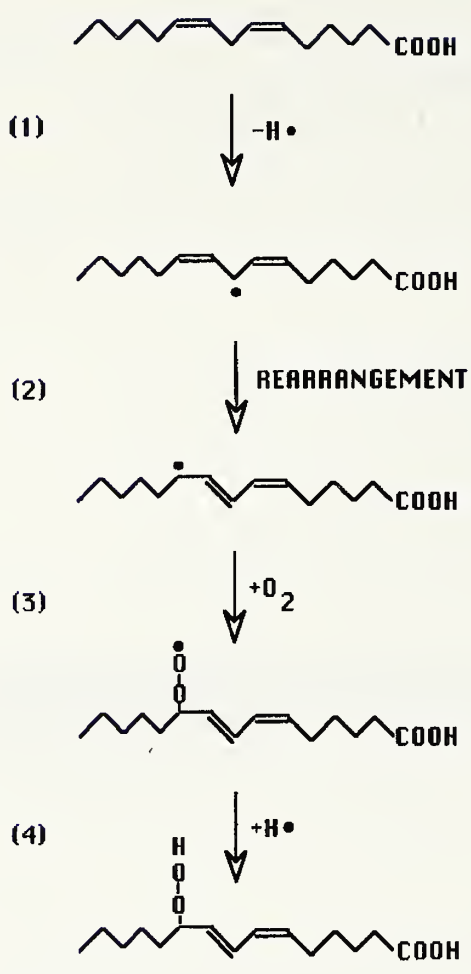

FIGURE 2

Reactions associated with lipid peroxidation. Reactions 1 and 2 represent the initiation of lipid peroxidation, when a hydrogen atom is abstracted from a lipid, and rearrangement of the resulting free radical to a more stable conformation. Reactions 3 and 4 represent the propagation of lipid peroxidation. After uptake of an oxygen atom, the free radical can abstract a hydrogen atom from another lipid, forming another free radical and a lipid hydroperoxide.

hydroperoxy (ROO·) or alkoxy (RO·) radicals. The latter reaction is similar to the Fenton reaction (reactions 4 and 5). For example, the hydroperoxide can react with Fe(II) to form Fe(III), hydroxide ion, and the alkoxyl radical.

$$ROOH + Fe^{2+} = = \Rightarrow Fe^{3+} + OH^{-} + RO\cdot \quad (6)$$

The alkoxyl radical can then initiate further lipid peroxidation chain reactions.

The relative reactivity of free radicals to induce lipid peroxidation is as follows: hydroxyl (·OH) > alkoxyl (RO·) > peroxyl (ROO·) > superoxide (O_2^-). The superoxide radical is least effective because it tends to remain in the cytosol and does not easily permeate to the lipid regions of membranes.

Besides the formation of lipid hydroperoxides, peroxyl radicals can cyclize to form cyclic peroxides and cyclic endoperoxides. Cyclic endoperoxides can be cleaved in the presence of oxygen molecules to form hydrocarbons, aldehydes, and shorter-chained fatty acids. The aldehydes can react with the amino- and sulfhydryl groups of proteins to produce cross-linking between and within the molecules (Wolff et al. 1986). Such reactions have been associated with the inactivation of enzymes, and if enough of them occur, the result can be the complete loss of membrane integrity and cell death.

Lipid hydroperoxides are found in the brain (Noda et al. 1983). Their distribution is slightly variable, with the highest concentrations detected in the substantia nigra and lowest concentrations in the corpus callosum.

ENZYMES THAT REDUCE THE TOXICITY OF FREE RADICALS

All biological systems possess the means to remove free radicals produced by reactive oxygen metabolites. Two general reactions remove these reactive metabolites: the reduction of the superoxide radical to hydrogen peroxide and oxygen, and

the reduction of hydrogen peroxide to water.

The reduction of the superoxide radicals to hydrogen peroxide is catalyzed by the enzyme superoxide dismutase (SOD). Located in the cytosol, SOD is a copper- and zinc-containing enzyme that catalyzes reaction 7.

$$O_2^- + O_2^- + 2H^+ = = \Rightarrow H_2O_2 + O_2 \quad (7)$$

The Zn(II) ion stabilizes the enzyme complex. The copper ion is located in the catalytic site and serves as a temporary source of electrons for the dismutation reaction. One of the electrons of the Cu(II) ion is transferred by this reaction to the superoxide radical, forming molecular oxygen. In the presence of the reduced copper ion, Cu(I), hydrogen ions (protons) combine with other superoxide radicals to form hydrogen peroxide. The extra electron is then transferred back to the copper ion.

$$SOD\text{-}Cu^{2+} + O_2^- = = \Rightarrow SOD\text{-}Cu^+ + O_2 \quad (8)$$

$$SOD\text{-}Cu^+ + O_2^- + 2H^+ = = \Rightarrow SOD\text{-}Cu^{2+} + H_2O_2 \quad (9)$$

As in other organs studied, SOD in the brain is found predominantly in the cytosol and has enzymatic activity similar to other organs (see review of Fried 1979). The regional distribution of SOD is similar throughout the brain. However, glial cells have activity six times higher than that in neurons.

The main pathway by which cells remove hydrogen peroxide is catalyzed with glutathione peroxidase, found predominately in the cytosol. This pathway uses glutathione (GSH), a tripeptide composed of glutamate, cytosine, and glycine, as a hydrogen donor to reduce hydrogen peroxide to water while converting GSH to its oxidized form (GSSG) as shown in reaction 10.

$$H_2O_2 + 2GSH = = \Rightarrow GSSG + 2H_2O \quad (10)$$

The element selenium is part of the active site of glutathione peroxidase, bound in the proteins as the amino acid selenocysteine (with selenium replacing the sulfur atom).

To have sufficient amounts of GSH to remove hydrogen peroxide, glutathione reductase maintains a high GSH/GSSG ratio by catalyzing the reduction of GSSG to GSH with reduced nicotinamide adenine dinucleotide phosphate (NADPH). The pentose phosphate pathway is an important source of the NADPH that serves as the hydrogen donor.

Although less abundant than superoxide dismutase, glutathione peroxidase is present in brain. Regional distribution studies have found that glutathione peroxidase and selenium are highest in the caudate-putamen and the substantia nigra (Brannan et al. 1980; Larsen et al. 1979) where dopamine is synthesized. Metabolism of dopamine by monoamine oxidase results in the production of hydrogen peroxide, which is coupled to oxidation of GSH (Maker et al. 1981). In

the cerebrum, glutathione peroxidase is found in high concentrations in glial cells (Savolainen 1978).

Hydrogen peroxide can also be converted to harmless metabolites by the enzyme catalase. However, this enzyme appears to play a significant role only when hydrogen peroxide concentrations are high. Catalase activity is generally associated with subcellular particles known as peroxisomes and catalyzes the reaction during which hydrogen peroxide is converted to water and oxygen.

$$2H_2O_2 = = \Rightarrow 2H_2O + O_2 \qquad (11)$$

Fe(III) is bound to the active site of catalase, but the exact manner in which it participates in the reaction is unclear. However, the formation of an intermediate called Compound I is involved. Compound I is fairly reactive and can initiate free radical formation.

In addition to high concentrations of catalase in the liver and erythrocytes, several studies have suggested the presence of catalase in trace amounts in the brain. The enzyme itself has been identified both by biochemical and histologic techniques and is located in microperoxisomes in neuronal cell bodies (Gault and DeDuve 1976; McKenna et al. 1976). In addition, the formation of hydrogen peroxide has been demonstrated in the brain and can be enhanced in the presence of the catalase inhibitor 3-amino-1,2,4-triazole (Sinet et al. 1980).

EVIDENCE OF POSSIBLE FREE RADICAL DAMAGE AFTER ETHANOL ADMINISTRATION

The ability of ethanol to enhance the formation of free radicals and induce lipid peroxidation has not been widely studied. However, a few reports have described conditions under which these processes could occur. Initially, investigations looked for indirect evidence of free radical damage or for changes in the protective antioxidant systems after chronic exposure to ethanol (see Hunt 1985; Nordmann et al. 1992).

Lipid peroxidation has been associated with the formation of lipofuscin pigments that appear as cytoplasmic granules in the brain (Côté 1981). These pigments are believed to be reaction products of peroxidized lipids and denatured proteins (Mead 1976). Chronic ethanol administration to rats for up to 18 months and subsequent withdrawal for 6 months has been reported to accelerate the formation of lipofuscin in the cerebellum, hippocampus, and prefrontal cortex (Freund 1979; Tavares and Paula-Barbosa 1983; Tavares et al. 1985; Borges et al. 1986; Cadete-Leite et al. 1988; see also Pentney, chapter 12). These results suggest the presence of free radical damage.

Little evidence exists suggesting that the enzymatic systems that normally minimize the concentrations of free radicals in brain tissue are impaired after ethanol exposure. Short-term chronic ethanol exposure has been reported to inhibit superoxide dismutase in rat brain (Ledig et al. 1981). However, only small changes were found in postmortem brain tissue from alcoholics (Marklund et al. 1983). Zinc and copper concentrations in brains from human alcoholics have been reported to be lower (Kasarskis et al. 1985). Moreover, acute and chronic administra-

tion of ethanol can decrease brain concentrations of zinc and copper in rats (Yamaoka 1989; Houzé et al. 1991). However, zinc concentrations are unchanged in chronic ethanol-treated rats when adequate amounts of the ion are provided in the diet (Kasarskis et al. 1985).

Although a single dose of ethanol can elevate lipid hydroperoxide levels and reduce glutathione levels in rat brain homogenates, no effects are observed after chronic ethanol treatment (Uysal et al. 1986, 1989). On the other hand, blood selenium concentrations in alcoholics are significantly lower than controls even with adequate nutrition and liver function (Dworkin et al. 1984). No studies have reported on possible changes in glutathione peroxidase in brain after ethanol administration.

Finally, acute and chronic administration of ethanol increases the concentrations of free iron in the rat brain (Yamaoka 1989; Rouach et al. 1990). Such a change could enhance the Fenton reaction (reaction 4) and lead to an elevation in hydroxyl radical reactions.

POSSIBLE SOURCES OF FREE RADICALS AFTER ETHANOL CONSUMPTION

Free radicals could form after ethanol consumption by several mechanisms. Some are covered in detail in other chapters and will be discussed only briefly here.

The metabolism of ethanol in the brain is a possible source of free radicals. Although it is accepted that the brain has low alcohol dehydrogenase activity (Raskin and Sokoloff 1968), there is evidence that ethanol can be metabolized by other means. Both ethanol-inducible cytochrome P450 and catalase have been found in the brain (Tindberg et al. 1989; Cohen et al. 1980) and are involved in the generation of free radicals in both the brain and liver (Ingelman-Sundberg and Johansson 1984; Cohen et al. 1980). Ethanol does not appear to alter catalase activity (Aragon et al. 1991), although an action of catalase on ethanol may become an important source of free radicals.

One interesting source of free radicals is the ethanol molecule itself. The α-hydrogen on the ethanol molecule can be abstracted by a hydroxyl radical to form the hydroxyethyl radical by the following reaction.

$$CH_3CH_2OH + \cdot OH = = \Rightarrow CH_3\cdot CHOH + H_2O \quad (12)$$

Using a brain microsomal fraction and a spin-trapping agent, which binds free radicals and renders them stable for detection by electron paramagnetic resonance spectroscopy, hydroxyethyl radicals have been detected in the presence of ethanol (20 to 85 mM) (Gonthier et al. 1991) in a concentration-dependent manner (Ahmad et al. 1988). Hydroxyethyl radicals are less reactive than hydroxyl radicals and can react with each other to form 2,3-dimethyl-1,4-butanediol (Halliwell and Gutteridge 1989). Further evidence that hydroxyethyl radicals form after ethanol exposure might be the detection of 2,3-dimethyl-1,4-butanediol in the brain. This compound would be more stable than the radicals. For a further discussion

of the effect of free radicals on physiological function, see Pellmar (chapter 16).

Finally, a source of free radicals could come from the accumulation of nitric oxide. Nitric oxide is a gaseous free radical that acts like a neurotransmitter (Snyder 1992) and may mediate physiological processes associated with learning and memory, such as long-term potentiation (Schuman and Madison 1991) and excitotoxicity (Dawson et al. 1991). However, the damaging species may not be nitric oxide. For example, nitric oxide can combine with the superoxide radical to form peroxynitrite (Snyder 1992). Peroxynitrite, in turn, decomposes to hydroxyl and nitroxide free radicals, which are much more reactive than nitric oxide. A role for nitric oxide in the excitotoxic actions of ethanol is currently being explored (Crews and Chandler, chapter 17; Lancaster, chapter 18).

FREE RADICAL SCAVENGERS AS POSSIBLE TREATMENT FOR BRAIN DAMAGE

Concentrations of free radicals are controlled not only by the enzymatic mechanisms discussed above but also by antioxidants. The two main antioxidants in the brain are ascorbate (vitamin C) and α-tocopherol (vitamin E) (Siegel et al. 1989). Ascorbate, an unsaturated sugar derivative, is a strong reducing agent and participates as a cofactor in the synthesis of catecholamines. α-Tocopherol is a lipid-soluble isoprenoid linked to a double-ring system, and it can intercept reactive intermediates in the formation of lipid hydroperoxides from polyunsaturated fatty acids in membranes. The vitamin may even have efficacy in treating cerebral ischemia (Hara et al. 1990).

Ethanol can alter the concentrations and availability of antioxidants in the brain. Ascorbate and α-tocopherol concentrations have been reported to be reduced in the cerebellum after acute and chronic ethanol administration (Rouach et al. 1987, 1991). In addition, serum concentrations of α-tocopherol in alcoholics are also decreased (Majumdar et al. 1983; Bjørneboe et al. 1988). These results suggest that decreases in the concentrations of these vitamins may play a role in ethanol-induced oxidative stress. In addition, replenishing these vitamins may have efficacy in reversing brain damage or minimizing further damage.

The few studies with experimental animals using antioxidants to prevent brain damage or associated cognitive deficits have yielded mixed results. Although diets fortified with vitamin E blocked ethanol-induced accumulation of lipofuscin in the brain, the learning deficits are not prevented (Freund 1979). Vitamin E and piracetam, another antioxidant, prevent ethanol-induced increases in lipid peroxidation in the brain (Nadiger et al. 1988) and the formation of lipofuscin in the cerebellum and hippocampus (Paula-Barbosa et al. 1991). However, these latter studies did not report behavioral measures.

Iron chelators also are efficacious in diminishing free radical-related effects. Desferrioxamine reduces the severity of ethanol withdrawal seizures (Abu-Murad and Nordmann 1983). Allopurinol,

which decreases the ability of xanthine oxidase to induce oxidative stress, prevents the reductions in selenium, zinc, and copper induced by ethanol (Houzé et al. 1991).

CONCLUSIONS

The preceding discussion and the following chapters suggest that ethanol-induced formation of free radicals contributes to the development of brain damage during long-term exposure to ethanol. However, they do not provide unequivocal results that ethanol exposure will not only lead to the formation of significant concentrations of free radicals in the brain but will also lead to consequent damage to cells.

Both direct and indirect evidence suggest that these radicals form and that the enzymatic machinery involved in maintaining low levels of them is impaired under some experimental conditions. However, the effects observed are found largely after acute exposure, not chronic exposure. This finding suggests that the brain can adequately protect itself during long-term ethanol exposure against some free radicals.

Much of the literature on free radicals assumes that free radicals are causative factors in the ultimate damage incurred after exposure to a toxin. Unfortunately, since brain homogenates undergo more lipid peroxidation than intact tissue (Halliwell and Gutteridge 1989), it is difficult to show conclusively that ethanol-induced increases in free radicals are not due to cell injury in the preparation. As a result, it is not clear whether the free radicals caused the cellular damage or resulted from it. Further research will possibly clarify these issues.

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16

DO FREE RADICALS CONTRIBUTE TO ETHANOL-INDUCED SYNAPTIC DAMAGE?

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INTRODUCTION

Increasing evidence suggests that free radicals contribute to the actions of ethanol in the brain. Specifically, brain microsomes have been found to produce hydroxyethyl radicals with exposure to ethanol (Gonthier et al. 1991). Hydroxyethyl radicals are created when ethanol interacts with hydroxyl radicals derived from hydrogen peroxide (see Hunt, chapter 15). They also may be produced through a cytochrome P450-mediated oxidation reaction or during the metabolism of ethanol to acetaldehyde (see Nordmann et al. 1992). However, neither of these reactions has been demonstrated in the brain.

Acute exposure to ethanol produces lipid peroxidation, a consequence of free radical exposure, in the brain (Nordmann et al. 1990; Uysal et al. 1989). Decreases in superoxide dismutase, α-tocopherol, ascorbate, and selenium occur following acute exposure (Houzé et al. 1991; Ledig et al. 1981; Nordmann et al. 1990). Moreover, ethanol has been found to reduce glutathione levels in brain homogenates (Nordmann 1990, 1992; Uysal et al. 1989) but not glutathione peroxidase (Houzé et al. 1991).

After chronic exposure to ethanol, lipid peroxidation is evident in the cerebellum (Nordmann et al. 1990) but not in whole brain homogenates (Uysal et al. 1989). In addition, chronic ethanol can decrease levels of certain antioxidants (Guerri and Grisolia 1980; Nordmann et al. 1990).

The neurophysiological consequences of free radical formation need to be investigated. This chapter will review current research on the electrophysiological effects of radicals in neuronal systems and compare them with the known actions of ethanol. Similarities might provide a rationale for further exploring the role of free radicals in mediating ethanol's actions.

CHRONIC VERSUS ACUTE EFFECTS OF ETHANOL AND FREE RADICALS

Electrophysiological studies on free radical actions in neural tissue have focused

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primarily on acute effects. The methods for generating free radicals (see below) limit our capability of controllably generating for days or months these short-lived, reactive compounds. Although ethanol may generate free radicals with chronic as well as with acute exposure (see Hunt, chapter 15), the free radical effects over a long timecourse may be quite different from acute effects. These differences may reflect adaption to oxidative stresses. For these reasons, this chapter will confine itself to the comparison of the acute effects of ethanol with acute actions of free radical exposure.

FREE RADICAL-GENERATING SYSTEMS

Hydroxyl free radicals can be formed from the reaction of hydrogen peroxide with iron and/or copper (Fenton reaction) (see Hunt, chapter 15). These radicals are perhaps the most damaging of the free radical species (Halliwell and Gutteridge 1989). Whereas peroxide is freely permeable through the cell membrane, the hydroxyl radical is formed at specific sites (Chevion 1988) associated with reactive metals (e.g., Fe or Cu). There are sufficient concentrations of metals in neurons for this reaction to occur and generate free radicals (Halliwell and Gutteridge 1985, 1989). Exposure of hippocampal neurons to hydrogen peroxide decreases electrophysiological excitability, an effect almost eliminated by treatment of the tissue with the iron chelator desferal (Pellmar et al. 1989). This finding suggests that peroxide itself is not the damaging agent but rather the product of the reaction of peroxide and iron—that is, hydroxyl radicals.

Another species of active oxygen, superoxide, can be generated from either xanthine plus xanthine oxidase (Fridovich 1978) or dihydroxyfumarate (Halliwell 1977; Nilsson et al. 1969). With these systems, the free radicals are generated extracellularly. Although superoxide is thought to be permeable through anion channels (Halliwell and Gutteridge 1989), the extremely low resting chloride permeability of neurons would limit its entry. The damage elicited with this free radical-generating system, therefore, is limited by the lower oxidation potential of superoxide and its spatial constraints.

A variety of free radical species are created by exposure of biological systems to X- and γ-radiation (Casarett 1968). Free radicals and other active compounds are generated by radiation after deposition of energy in water molecules both intracellularly and extracellularly. This process can occur independent of cellular iron. However, the distribution of free radicals is different from the generating systems discussed above. Although water molecules, being predominant in biological tissue, are the main targets of radiation, cellular macromolecules might also be directly damaged by radiation exposure.

Ethanol can be quickly converted to hydroxyethyl radicals in the presence of hydroxyl radicals (Ahmad et al. 1988; Halliwell and Gutteridge 1989; see also Hunt, chapter 15). Adding ethanol to any of the other free radical-generating systems would likely generate this radical as well. The primary difficulty in evaluating the

hydroxyethyl radical is distinguishing its actions from those of the hydroxyl radical.

MEMBRANE PROPERTIES

The actions of free radicals and ethanol on basic cellular membrane properties have been evaluated in a number of preparations, including cells in tissue culture, brain slices, and isolated invertebrate ganglia. The effects of ethanol on the resting membrane potential are minimal, with only small and variable changes reported (Benson et al. 1989; Carlen et al. 1985; Frye et al. 1991; Gruol 1982; Niesen et al. 1988; Reynolds et al. 1990; Siggins et al. 1987*a*,*b*). In addition, the input resistance of the neurons is unaffected by exposure to ethanol (Benson et al. 1989; Frye et al. 1991; Gruol 1982; Niesen et al. 1988; Reynolds et al. 1990; Siggins et al. 1987*a*,*b*). A similar analysis of resting membrane properties has been done for hydrogen peroxide-generated hydroxyl radicals in hippocampal slices (Pellmar 1987) and in lobster cardiac ganglion neurons (Livengood 1988). In these cells, neither resting potential nor membrane resistance is changed. In contrast, many nonneuronal cells do respond to free radical exposure by depolarizing (Scott and Rabito 1988). These data suggest that actions of free radicals in neurons are more likely to be on active rather than passive electrical processes.

SPIKING ACTIVITY

Many investigators have reported that ethanol decreases both spontaneous (Carlen et al. 1982; Gruol 1982; Urrutia and Gruol 1992) and current-evoked (Benson et al. 1989; Carlen et al. 1991; Siggins et al. 1987*a*,*b*; Urrutia and Gruol 1992) firing of action potentials in a variety of neuronal types. However, at lower concentrations (10 to 22 mM) of ethanol, increases in spike frequency were sometimes observed (Niesen et al. 1988; Siggins et al. 1987*a*,*b*; Urrutia and Groul 1992). In cultured spinal cord neurons showing bursting activity, burst frequency slows but single spikes increase (Gruol 1982).

Peroxide-generated free radicals decrease repetitive firing elicited by a depolarizing current in CA1 hippocampal pyramidal cells (Pellmar 1987). In addition, in neurons of the lobster cardiac ganglion, peroxide disrupts the normally regular bursting pattern (Hobbs et al. 1988).

In the hippocampal slice preparation, stimulation of an afferent pathway orthodromically (from cell body to synapse) activates an identifiable population of neurons. The synchronous synaptic potentials and action potentials (population spikes) can be recorded extracellularly. The relationship between the size of the excitatory postsynaptic potential (EPSP) and the population spike amplitude reflects the spike-generating capability of the neurons. This relationship is known as EPSP/spike (E/S) coupling.

All the free radical-producing systems tested (X- and γ-radiation, peroxide, and dihydroxyfumarate) decrease E/S coupling (Pellmar 1986; Pellmar and Lepinski 1992; Pellmar et al. 1990; Tolliver and Pellmar 1987). In one study in which ethanol (66 mM) was used as a solvent control, E/S coupling was resistant, showing only a small enhancement of this rela-

tionship (Pellmar 1991). Siggins et al. (1987*b*) reported that the spike threshold recorded intracellularly with afferent stimulation was increased following 10 to 15 mM ethanol, which would be a reflection of decreased E/S coupling.

The amplitude of the population spike is affected by both E/S coupling and the size of the synaptic potential. Several studies have shown that the amplitude of the population spike is decreased by ethanol at concentrations of 40 mM or more (Sellin and Laakso 1987; Siggins et al. 1987*b*). On the other hand, Takada et al. (1989) reported that the population spike is resistant to ethanol up to 140 mM. Most of the free radical-generating systems (dihydroxyfumarate, hydrogen peroxide, and γ-radiation) decrease the population spike (Pellmar 1986; Pellmar and Lepinski 1992; Tolliver and Pellmar 1987). With X-radiation at low dose-rates, in contrast, the population spike is generally increased, because synaptic transmission is enhanced (Pellmar et al. 1990).

Taken together, the evidence presented in this section suggests that exposure to either ethanol or free radical-generating systems generally suppresses actions potentials.

THE AFTERHYPERPOLARIZATION

The frequency of firing of action potentials can be modulated through changes in the calcium-dependent potassium current underlying the spike afterhyperpolarization (AHP) (Madison and Nicoll 1984). The reported effects of ethanol on this potential are inconsistent. Some studies (Carlen et al. 1982, 1985; Niesen et al. 1988; Reynolds et al. 1990) indicated that the AHP is enhanced by ethanol at concentrations as low as 20 mM in Purkinje cells, hippocampal pyramidal cells, and young rat dentate granule cells. Enhancement of the AHP could explain the frequently observed decrease in firing frequency. In contrast, other laboratories (Siggins et al. 1987*a*,*b*; Taube and Schwartzkroin 1986; Urrutia and Gruol 1992) reported a decrease or no change in the AHP.

The issue is not resolved with voltage-clamp experiments that directly measure the outward potassium current corresponding to the AHP. Madsen and Edeson (1990) reported that 10 to 50 mM ethanol increases this calcium-dependent potassium current in molluscan neurons, whereas other groups (Benson et al. 1989; Moore et al. 1990) saw little effect on the current in hippocampal pyramidal cells. Free radicals generated from hydrogen peroxide do not alter the AHP or a calcium-dependent potassium current in hippocampal pyramidal cells (Pellmar 1987).

OTHER POTASSIUM CURRENTS

Other potassium currents can also influence firing frequency of neurons (Connor 1980; Madison and Nicoll 1984). A decrease in potassium currents would, in general, increase excitability of the neurons. However, the data show only limited effects on these currents. For example, a muscarine-sensitive current (M-current) is reduced by 22 to 44 mM ethanol (Moore et al. 1990), yet an early transient outward current (A-current) is unaffected (Madsen and Edeson 1990; Moore et al.

1990) except at concentrations greater than 200 mM (Treistman and Wilson 1987). The delayed rectifier (a sustained outward potassium current) is also unaffected by 50 mM ethanol in *Helix* neurons (Madsen and Edesen 1990).

Similarly, peroxide does not affect either the A-current or the delayed rectifier in both hippocampal and lobster cardiac ganglion neurons (Livengood 1988; Livengood and Miller 1989; Pellmar 1987). In hippocampal pyramidal cells, M-current is equally insensitive to peroxide. However, in cardiac myocytes, dihydroxyfumarate-generated superoxide (Cerbai et al. 1991) and reactive oxygen resulting from photoactivation of rose bengal (Tarr and Valenzeno 1991; Valenzeno and Tarr 1991) reduced the outward rectifying potassium current.

CALCIUM

Ethanol can decrease calcium inward currents in a variety of neurons. Concentrations of 30 mM and above decreased both the transient (T) and sustained (L) inward currents in neuroblastoma cells (Twombly et al. 1990) as well as the inward currents in *Aplysia* (Camacho-Nasi and Treistman 1986, 1987). However, Treistman et al. (1991) observed a greater sensitivity of the L-current than the T-current in the nerve terminals of the neurohypophysis. In dentate granule cells of the hippocampus, Carlen et al. (1991) observed decreases in T- and L-currents at room temperature but not when the tissue was warmed to 30 °C, where intracellular mechanisms to regulate calcium are likely to be more active.

In lobster cardiac ganglion neurons, Livengood (1988, 1989) observed that peroxide blocked calcium inward currents. The transient inward current, which shows a variable delay in onset and may be a calcium spike from an unclamped region of the cell (Miller et al. 1990), is very sensitive to hydrogen peroxide. The sustained inward current and the early transient inward current are also reduced but are less sensitive to the free radical insult. Keyser and Alger (1990) observed that xanthine/xanthine oxidase decreased sustained inward calcium currents in dissociated hippocampal neurons. Calcium inward currents in cardiac myocytes are similarly sensitive to free radicals (Barrington et al. 1988; Goldhaber et al. 1989; Hayashi et al. 1989; Nakaya et al. 1987; Tarr and Valenzeno 1991; Valenzeno and Tarr 1991). The sensitivity to free radical-generating systems in these cells contrasts with the insensitivity of both the sustained and the early transient inward calcium currents recorded from hippocampal pyramidal cells in the slice preparation at 30 °C (Pellmar 1987, unpublished observation).

In general, both ethanol and peroxide inhibit inward calcium currents. However, the involvement of a particular calcium subtype is still unclear.

SYNAPTIC TRANSMISSION

The reported effects of ethanol on synaptic potentials are inconsistent. No changes in inhibitory postsynaptic potentials (IPSPs) or EPSPs were observed with 20 to 30 mM ethanol in cultured spinal neurons (Gruol 1982) or in hippocampal dentate granule

cells of young rats (Niesen et al. 1988). On the other hand, Siggins et al. (1987*a*,*b*) reported a decrease, whereas Carlen et al. (1982) reported an increase, in EPSPs and IPSPs of CA1 pyramidal cells of rat hippocampus. At the crayfish neuromuscular junction, no changes in inhibitory or excitatory synaptic potentials were observed with concentrations up to 90 mM (Blundon and Bittner 1992), although decreases occurred with higher concentrations (Friedman et al. 1988; Blundon and Bittner 1992).

The effects of free radicals on synaptic potentials depend on the generating system. Both γ-radiation and hydrogen peroxide decrease synaptic potentials recorded extracellularly in hippocampal slices (Pellmar 1986; Tolliver and Pellmar 1987). Intracellular recording reveals that the inhibitory as well as the excitatory postsynaptic potentials are affected by exposure to peroxide (Pellmar 1987). Peroxide is also effective in decreasing the excitatory synaptic potential at the lobster neuromuscular junction and squid giant synapse (Colton et al. 1986, 1991; Colton and Gilbert 1985). Superoxide generated from dihydroxyfumarate does not affect synaptic transmission in hippocampal neurons (Pellmar and Lepinski 1992). This inconsistency may be related to the extracellular localization of the free radical in this system. In the presence of superoxide dismutase, which would enhance the production of peroxide, dihydroxyfumarate reduces synaptic transmission (Pellmar and Lepinski 1992). At the squid giant synapse and the lobster neuromuscular junction, superoxide generated with xanthine/xanthine oxidase also depresses synaptic transmission (Colton et al. 1991). On the other hand, X-radiation at low dose-rates (1.5 Gy/min) increases synaptic potentials in field CA1 of hippocampus (Pellmar et al. 1990). This enhancement has not been mimicked by other free radical-generating systems but can be replicated by exposure to free fatty acids such as oleic acid or arachidonic acid (Linden et al. 1986; Pellmar 1991; Williams et al. 1989). Release of fatty acids occurs with lipid peroxidation (Borowitz and Montgomery 1989; Chan et al. 1984; van Kuijk et al. 1987) and may mediate some electrophysiological damage initiated by free radicals.

Use of synaptosomes allows one to look at the release of neurotransmitters from presynaptic terminals. Ethanol reduces the release of the amino acid neurotransmitters only at concentrations greater than 100 mM (Carmichael and Israel 1975; Clark and Dar 1989; Rohde and Harris 1983; Strong and Wood 1984; Suzdak et al. 1986). In contrast, synaptosomal glutamate release (Gilman et al. 1992) is depressed by exposure to peroxide, whereas amino acid transmitter release is enhanced in a hippocampal slice preparation (Pellegrini-Giampietro et al. 1990; Borchert et al., personal communication). The differences between synaptosomes and slices could be due to radical-induced impairment of reuptake mechanisms by glial cells present in the slice preparation.

POSTSYNAPTIC RESPONSES TO AMINO ACID TRANSMITTERS

γ-Aminobutyric Acid

The sensitivity of γ-aminobutyric acid (GABA)-evoked inhibitory responses to

ethanol has received a great deal of attention (see also Hunter, chapter 13; Shanley and Wilce, chapter 14). Many studies have indicated that ethanol can increase chloride flux accompanying stimulation of GABA receptors (Engblom et al. 1991; McQuilkin and Harris 1990; Mehta and Ticku 1990; Suzdak et al. 1986) and increase the electrophysiological response to GABA application (Aguayo 1990; Celentano et al. 1988; Givens and Breese 1990; Nestoros 1980; Nishio and Narahashi 1990). Yet, many other studies found that ethanol does not significantly affect the inhibitory actions of GABA (Carlen et al. 1982; Frye et al. 1991; Gruol 1982; Mihic et al. 1992; Osmanovic and Shefner 1990; Siggins et al. 1987*a*,*b*; White et al. 1990). In fact, Aguayo (1990) observed some neuronal selectivity for the actions of ethanol. Whereas the GABA-induced currents in cortical neurons and most hippocampal neurons were potentiated by ethanol, the responses in some hippocampal neurons were insensitive. The requirement for an unidentified factor (McQuilkin and Harris 1990; Mihic et al. 1992); differences in metabolic state, lipid content, and $GABA_A$-receptor subtype (White et al. 1990); and specific experimental conditions (McQuilkin and Harris 1990) have also been suggested as explanations for the discrepancies. The GABA response in hippocampal pyramidal cells is not sensitive to hydrogen peroxide-generated free radicals (Pellmar 1987).

Glutamate

In an early study (Gruol 1982), the response to iontophoretic application of the excitatory amino acid glutamate was insensitive to ethanol (20 to 80 mM). More recently, the various glutamate receptor subtypes (named for their specific agonists) have been analyzed for their sensitivity (see Crews and Chandler, chapter 17). The N-methyl-D-aspartate (NMDA) receptor is frequently associated with a voltage-dependent inward current accompanied by an influx of calcium ions. Activation of the non-NMDA receptors (kainate and quisqualate) does not show the same voltage dependence. At resting membrane potential, non-NMDA receptors predominate in the response to glutamate.

Non-NMDA receptors are not very sensitive to ethanol (Lovinger et al. 1989, 1990*b*; Martin et al. 1991), requiring approximately 170 mM to produce a 50-percent block (Martin et al. 1991). In contrast, the response to NMDA is exquisitely sensitive to ethanol (Lima-Landman and Albuquerque 1989; Lovinger et al. 1989, 1990*b*; Martin et al. 1991; Simson et al. 1991; Weight et al. 1991; White et al. 1990), with a 50-percent decrease occurring with ethanol concentrations between 10 and 50 mM, depending on the preparation (Lovinger et al. 1990*b*; Martin et al. 1991; Weight et al. 1991; White et al. 1990). However, at very low concentrations, some potentiation of the NMDA-evoked current may occur (Lima-Landman and Albuquerque 1989; White et al. 1990).

At the single channel level, the probability of channel opening increases at very low ethanol levels and decreases at higher concentrations, with a concomitant decrease in the average time the channel

remains open (Lima-Landman and Albuquerque 1989).

The sensitivity of the NMDA receptor is seen with endogenous neurotransmitter in the hippocampal slice preparation (Lovinger et al. 1990*a*,*b*; Weight et al. 1991). The synaptic potential can be pharmacologically manipulated (low magnesium and block of non-NMDA receptors) so that it is predominantly mediated through the NMDA receptor. This potential is readily blocked by ethanol, in contrast to the non-NMDA mediated synaptic potential, which is inhibited only 9 percent by 100 mM ethanol (Lovinger et al. 1990*a*; Weight et al. 1991). The limited action of ethanol on the non-NMDA-mediated synaptic potential is consistent with the limited effects of ethanol on the EPSP (see above).

Acute or prolonged exposure to free radicals does not affect the response to iontophoresed glutamate onto hippocampal pyramidal cells (Pellmar 1987). The response to exogenous glutamate at the lobster neuromuscular junction (Colton et al. 1986; Colton and Gilbert 1985) is also unchanged by peroxide exposure. Yet, the superoxide-generating system xanthine/xanthine oxidase decreases responses to NMDA in rat cortical neurons (Aizenman et al. 1990). As a result, a redox modulatory site on the NMDA receptor has been postulated (Aizenman et al. 1989, 1990). This conclusion is also based on the observation that the reducing agent dithiothreitol upregulates receptor sensitivity, an effect reversed by superoxide radicals and by the oxidizing agent 5,5-dithio-bis-2-nitrobenzoic acid.

The NMDA receptor has several regulatory sites being investigated as targets for ethanol and free radicals. Most studies report that the actions of ethanol are independent of NMDA concentration (Dildy-Mayfield and Leslie 1991; Martin et al. 1991; Weight et al. 1991), suggesting that ethanol does not compete for the neurotransmitter binding site. Hoffman et al. (1989), however, did report a competitive interaction.

Magnesium antagonizes NMDA-induced current by binding at a site in the associated channel. The effectiveness of magnesium was unchanged in the presence of ethanol in several studies (Dildy-Mayfield and Leslie 1991; Rabe and Tabakoff 1990; Weight et al. 1991; Woodward and Gonzales 1990), but not all (Martin et al. 1991).

The effects of ethanol at the glycine binding site, an allosteric co-agonist binding site, are controversial. There are several reports (Dildy-Mayfield and Leslie 1991; Hoffman et al. 1989; Rabe and Tabakoff 1990; Woodward and Gonzales 1990) that additional glycine can inhibit the effects of ethanol at the NMDA receptor, whereas others (Martin et al. 1991; Peoples and Weight 1992; Weight et al. 1991) find that glycine and ethanol are not competitive. A new hydrophobic site in the receptor has been suggested (Lovinger et al. 1989; Weight et al. 1991) because of the correlation between the effectiveness of various alcohols at the NMDA receptor and their hydrophobicity. The exact site for ethanol effects on the NMDA receptor remains unresolved.

Less work has been done with the NMDA redox-modulatory site. Aizenman

et al. (1989) ruled out an interaction at the agonist binding site, because the actions of the antagonist 2-amino-5-phosphonovalerate are unaltered by oxidation or reduction. In addition, they observed that magnesium inhibition and glycine potentiation occurred in both oxidized and reduced states. Tauck and Ashbeck (1990) reported a synergistic effect of glycine with the reducing agent dithiothreitol to enhance long-term potentiation (LTP), suggesting that the glycine binding site may be a locus of interaction.

LONG-TERM POTENTIATION

Several investigators (Blitzer et al. 1990; Mulkeen et al. 1987; Sinclair and Lo 1986; Taube and Schwartzkroin 1986) have observed that ethanol can impair LTP (see also Hunter, chapter 13). Blitzer et al. (1990) found a dose-dependent decrease in LTP at concentrations as low as 5 mM ethanol (figure 1). Other investigators (Mulkeen et al. 1987; Sinclair and Lo 1986; Taube and Schwartzkroin 1986) reported a similar decrease in LTP only with higher concentrations of ethanol (86 to 163 mM), reporting variable results at the lower concentrations (50 mM) (Sinclair and Lo 1986). The actions of ethanol persist in the presence of picrotoxin, suggesting that enhanced GABA inhibition is not the mechanism (Blitzer et al. 1990). A high correlation between the decrease in NMDA responses and the impairment of LTP suggests that ethanol's effects on LTP are a consequence of its actions on the NMDA receptor (Blitzer et al. 1990). Short-term potentiation is also decreased by ethanol, although with less sensitivity (Blitzer et al. 1990).

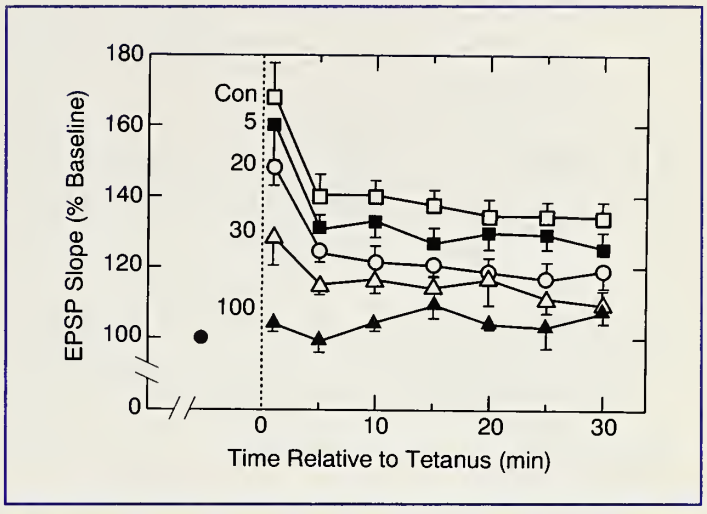

FIGURE 1

Effects of ethanol on LTP. LTP was initiated by high frequency stimulation at time = 0 (dashed, vertical line). The concentrations of ethanol used are to the left of this line. "Con" is the control potentiation in the absence of ethanol. EPSP slopes are relative to the pretetanus level (100 percent, filled circle). Reproduced with permission from Blitzer et al. (1990).

Free radicals also interfere with LTP (Colton et al. 1989; Pellmar et al. 1991). Concentrations of peroxide that have no measurable direct effect on orthodromic responses increase the rate of decay of LTP. By 60 minutes after stimulation, the responses in peroxide-treated tissue are almost back to the initial levels, whereas the responses in untreated tissue show sustained potentiation (Pellmar et al. 1991) (figure 2). In contrast to LTP, paired-pulse facilitation (Colton et al. 1989; Pellmar et al. 1991) and short-term potentiation (Pellmar et al. 1991) are unaffected by exposure to peroxide. The actions of free radicals and ethanol on LTP could share a common mechanism, that is, interaction with the NMDA receptor. Tauck and Ashbeck (1990) found that the reducing agent dithiothreitol enhanced potentiation and suggested modulation of the redox state of the

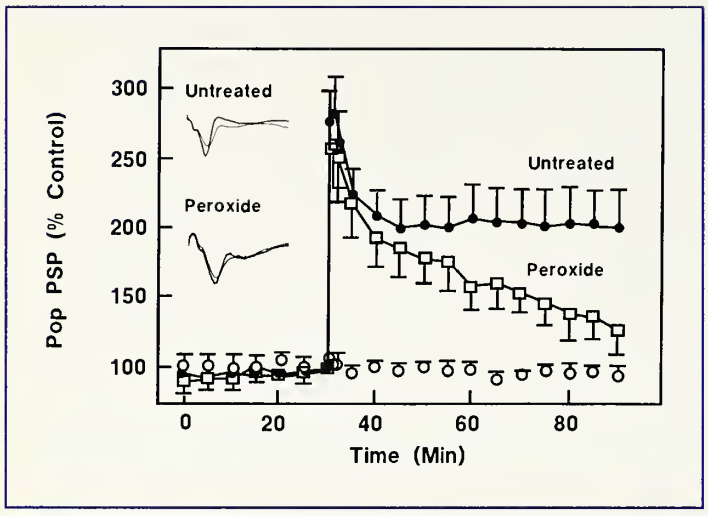

FIGURE 2

Effects of free radicals on LTP. LTP was initiated by high frequency stimulation at time = 30 minutes. EPSP slopes are relative to the prestimulation control (100 percent). Filled circles show LTP in the absence of free-radical-generating systems. Squares show LTP in the presence of peroxide used to generate hydroxyl radicals. Open circles show the absence of effects of peroxide without the high frequency stimulation. Insets show sample population synaptic potentials before high frequency stimulation (light trace) and 60 minutes after stimulation (dark trace) with and without peroxide present. Reproduced with permission from Pellmar et al. (1991).

NMDA receptor as a mechanism of modulation of LTP.

CONCLUSIONS

A comparison of free radical- and ethanol-induced actions on electrophysiological properties of neurons reveals many similarities, as well as some significant differences. Neither free radicals nor ethanol has strong effects on resting membrane properties. Moreover, both ethanol and free radicals have been shown to decrease calcium currents in some preparations. However, potassium currents differ in their sensitivity to ethanol and free radicals. Unlike ethanol, free radicals are very effective at decreasing amino acid transmitter release, probably through a presynaptic mechanism. Ethanol, but not free radicals, can enhance GABA-induced chloride currents, thereby increasing synaptic inhibition. Activation of NMDA receptors, a subclass of glutamate receptors, is antagonized both by ethanol and by free radicals. The impairment of the NMDA response may account for the actions of ethanol and free radicals to inhibit LTP.

The similarities of ethanol and free radical effects are intriguing and may suggest a role for the hydroxyethyl radical in ethanol-induced deficits. The differences are also striking and may reflect additional nonradical mechanisms. The contribution of hydroxyethyl radicals to the acute and chronic consequences of ethanol exposure needs further study.

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17

EXCITOTOXICITY AND THE NEUROPATHOLOGY OF ETHANOL

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INTRODUCTION

One of the pathological changes associated with chronic ethanol exposure is a reduction in neuronal density. This reduction may result from increases in neuronal intracellular calcium concentration ($[Ca^{2+}]_i$). Interestingly, these changes have been implicated in the neuronal loss accompanying aging, hypoxia, and chronic cerebral ischemia.

Recent studies show that excessive excitation of neurons can trigger a sequence of events that lead to an increase in $[Ca^{2+}]_i$ and eventually cell death. Increases in intraneuronal calcium can occur from uptake through voltage-dependent and receptor-operated calcium channels and from inositol 1,4,5-trisphosphate (Ins $1,4,5P_3$)-induced release of intracellular calcium. Other studies suggest that excitatory amino acid (EAA) transmitters, particularly glutamate, can increase $[Ca^{2+}]_i$ to a level that causes neuronal death. These neurotoxic actions of EAAs are called excitotoxicity (Cotman et al. 1989; Choi 1990; Clark 1989; Olney 1990). This chapter reviews various aspects of excitotoxicity and presents the hypothesis that the neuropathological actions of ethanol are due to sensitization of neurons to excitotoxicity during chronic ethanol exposure.

Glutamate and aspartate are the major neurotransmitters responsible for rapid excitatory neurotransmission as well as excitotoxicity. EAA-mediated excitotoxicity has been divided into two components: a rapid component, associated with osmotic swelling and often immediate neuronal death, and a delayed component, occurring as a progressive degenerative process over a period of hours to days. Both processes are likely due at least in part to the excessive accumulation of calcium (figure 1) (Meldrum and Garthwaite 1990; Clark 1989; Olney 1990).

In addition to calcium, the early process likely involves sodium (Na^+) flux through activated membrane channels, an increase in intracellular volumes, and a depletion of intracellular energy stores. The slow progressive neuronal degenera-

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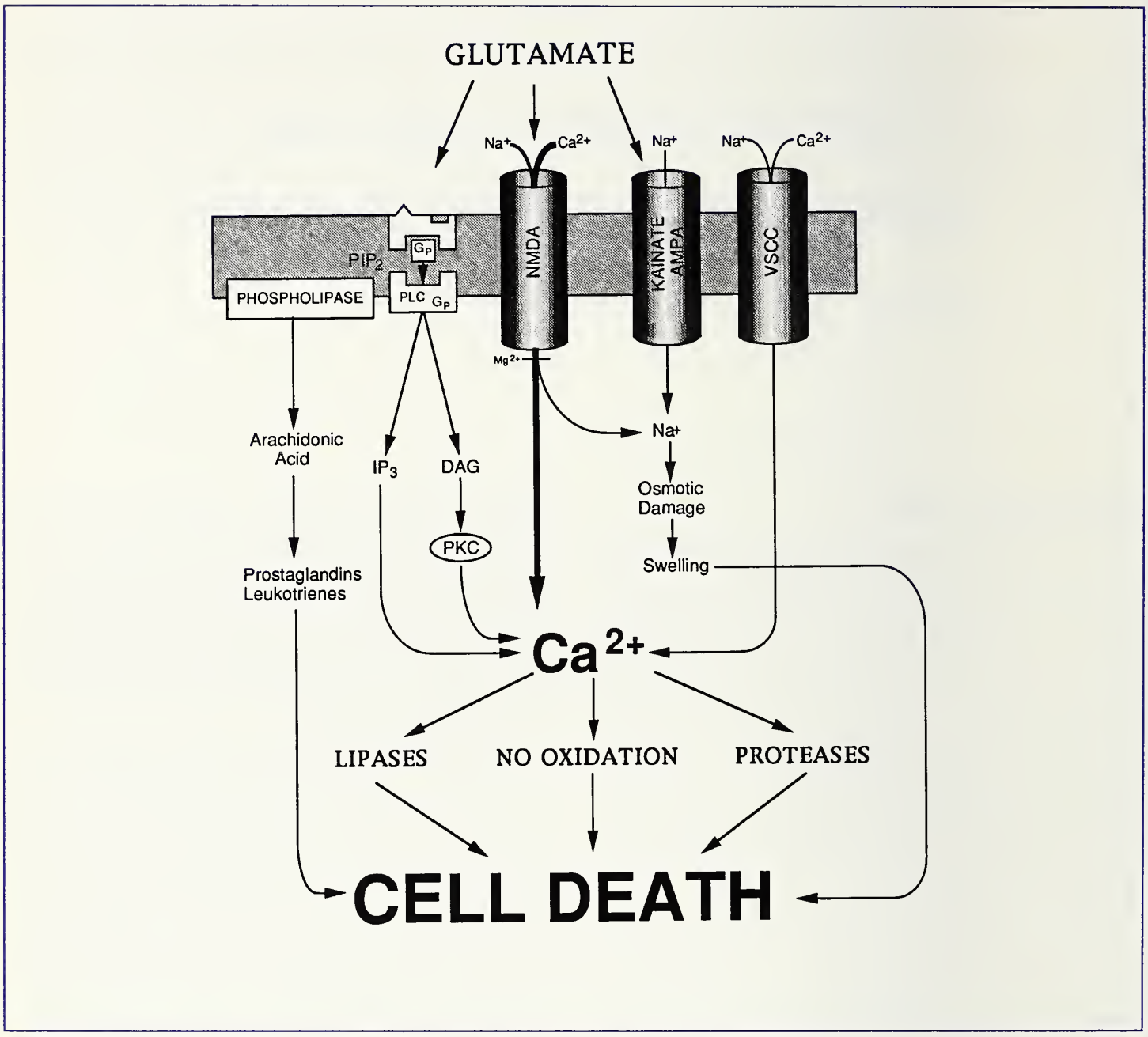

FIGURE 1

Schematic representation of excitatory amino acid-mediated excitotoxicity. Glutamate stimulates NMDA and kainate-AMPA receptors, depolarizes the neuron, and opens voltage-sensitive calcium channels. These events trigger processes that lead to neuronal cell death.

tion, that is, delayed neuronal cell death, appears to involve depolarization of neurons by glutamate, followed by increased passage of $[Ca^{2+}]_i$ through receptor-gated ion channels and voltage-sensitive calcium channels (Choi et al. 1988; Clark 1989).

Both glutamate and increased $[Ca^{2+}]_i$ activate phospholipases, generating Ins $1,4,5P_3$, which then releases intracellular calcium stores; diacylglycerol that activates protein kinase C (PKC); and arachidonic acid, which is metabolized by prostaglandin synthetases, generating free radicals and prostaglandins. Ultimately, additional proteases and endonucleases (enzymes that act on proteins and nucleic acids, respectively) are activated, leading to cell death (Orrenius et al. 1989).

Although a brief exposure to glutamate initially increases $[Ca^{2+}]_i$ even after

removal of glutamate, $[Ca^{2+}]_i$ remains increased throughout the progression to delayed neuronal death. This sustained increase in $[Ca^{2+}]_i$ appears to involve activation of PKC, since PKC inhibitors or downregulation of PKC have been reported to prevent the sustained increase in $[Ca^{2+}]_i$ and block delayed neuronal cell death (Favaron et al. 1990; Manev et al. 1989). Studies in vivo and in vitro suggest that the excitotoxic actions of glutamate are indicative of the general properties of all EAA receptors. Thus, excitotoxicity is a pathological mechanism, unique to the brain, that may be involved in a number of neurodegenerative processes.

EXCITATORY AMINO ACID RECEPTORS AND EXCITOTOXICITY

Pharmacological studies have distinguished at least four classes of glutamate receptors that molecular biology studies have shown are extremely heterogeneous. Three of the receptor classes are receptor-operated ion channels: N-methyl-D-aspartic acid (NMDA), kainate, and amino-3-hydroxy-5-methyl-4-isoxalone proprionic acid (AMPA). The fourth group of receptors is called metabotropic receptors, since they activate or inhibit second messenger systems such as phosphoinositide hydrolysis and adenylate cyclase activity. These processes are coupled through guanine nucleotide proteins. The excitatory potency of glutamate analogs generally parallels their excitotoxic potency, with NMDA and kainate being most potent.

Each of the pharmacological EAA receptor subtypes is heterogeneous (Monaghan et al. 1988; Cotman et al. 1989). NMDA receptors are characterized by their voltage-dependent block by extracellular magnesium, high Ca^{2+}/Na^{+} permeability ratio, requirement for glycine as a co-agonist, and slow onset and offset time courses of channel gating. The high influx of calcium during activation of NMDA receptors is critical for activity-dependent synaptic plasticity as well as playing a dominant role in excitotoxicity.

Several subtypes of the NMDA receptor are likely to exist (Nakanishi 1992; Michaelis et al. 1992). Molecular cloning has identified several different mRNA species that can form heteromeric ion channel structures (Monyer et al. 1992*b*). These subunits exhibit differential expression patterns in brain regions, suggesting that different heteromeric NMDA channels exist in various brain regions. Michaelis et al. (1992) have suggested that four or five proteins of different molecular weights combine to form functional NMDA channels. Although the exact diversity of the NMDA subtype of EAA receptors remains to be fully elucidated, NMDA receptors clearly play a key role in excitotoxicity.

Kainate and AMPA receptors are a heterogeneous group of ion channels composed of multiple subunits each of which has a unique pharmacology. There are multiple genes (GluR1-GluR7) that encode subunits, combining to form multisubunit AMPA-kainate receptors (Boulter et al. 1992). In general, GluR1-4 subunits group into AMPA-kainate receptors, whereas GluR5-7 tend to be predominantly kainate receptors. These ion

channels are permeant to sodium and potassium (Boulter et al. 1992; Monyer et al. 1992*a*), although in some instances, they do conduct calcium (Gilbertson et al. 1990). A major difference is that AMPA receptors readily desensitize during stimulation, whereas the response to kainate is prolonged with little or no desensitization. There are differences in rank order of potencies, ligand affinities, and distribution of binding sites in the brain that likely reflect multiple combinations of various subunits forming heteromeric ion channels with different agonist responses (Barnard et al. 1992).

GluR1 and GluR2 are the predominant mRNAs in the cortex and hippocampus. GluR2 has the unique property that one amino acid out of approximately 900 regulates calcium conductance for these receptor channels (Monyer et al. 1992*a*). Since calcium flux is a major determinant of excitotoxicity, changes in this subunit could tremendously influence glutamate-stimulated excitotoxicity for neurons not containing GluR2 subunits within their kainate-AMPA ion channels. The subunit composition and brain regional distribution of various subtypes of AMPA and kainate receptors that are particularly excitotoxic are not yet clearly delineated.

Different EAA agonists induce different patterns of neuronal degeneration. In hippocampal and cerebellar slices, NMDA induces a rapid and massive swelling and vacuolation of the cytoplasm of neurons with mitochondrial expansion and clumping of nuclear chromatin. Alternatively, AMPA and quisqualate induce a more gradual degeneration in which the somas and nuclei of vulnerable cells become progressively condensed and darkly stained. In addition, the cytoplasm becomes studded with microvacuoles; terminal dendritic fields show intense ballooning (Meldrum and Garthwaite 1990).

Cerebral cortical cell culture studies have found that glutamate neurotoxicity depends largely on activation of NMDA receptors. Direct exposure of cortical neurons in culture to NMDA for 3 to 5 minutes leads to massive and progressive neuronal death, whereas kainate exposure requires several hours to induce excitotoxicity (Choi et al. 1988). However, this may depend on several factors.

Certain neurons, relatively resistant to NMDA, have been found to be particularly sensitive to kainate (Koh and Choi 1988). This variability sometimes appears due to differences in receptor density. For example, kainate injected into rat hippocampus destroys CA3 neurons, which have a high density of kainate binding sites and are potently excited by kainate. On the other hand, cerebellar Purkinje cells in rats are resistant to NMDA excitotoxicity and express few NMDA receptors (Meldrum and Garthwaite 1990). Studies in brain slices have found an approximate 10-fold difference in sensitivity to NMDA excitotoxicity, with the most sensitive being CA1 hippocampal neurons > granule cells of the dentate gyrus > CA3 hippocampal neurons > granule cells of the cerebellum (Meldrum and Garthwaite 1990). These differences in sensitivity were observed for both dose and time of exposure to NMDA and are consistent with the distribution of NMDA receptors.

Studies with mouse cortical neurons in culture have found a small fraction of neurons that are resistant to NMDA-mediated excitotoxicity. These neurons were first identified using a histochemical stain for the enzyme nicotinamide adenine dinucleotide phosphate (NADPH)-diaphorase (Koh and Choi 1988). Recent studies have suggested that NADPH-diaphorase stains for nitric oxide synthase. Although production of nitric oxide is often associated with excitotoxicity (see below), there may be protective mechanisms in neurons containing nitric oxide synthase that protect them from excitotoxicity. Although the NADPH-diaphorase-stained neurons were less sensitive to NMDA excitotoxicity, they were particularly sensitive to kainate excitotoxicity.

Similarly, γ-aminobutyric acid (GABA) neurons identified by specific immunoreactivity represent approximately 10 percent of all neurons in cortical cultures and are also spared at concentrations of NMDA that kill 60 to 80 percent of neurons (Tecoma and Choi 1989). NADPH-diaphorase neurons may represent a subset of GABAergic cells, although these neurons are too few (1 to 2 percent) to account for all of the GABAergic neuron sparing. The intrinsic resistance of GABAergic neurons could be due to the presence of fewer NMDA receptors or because of some ability to withstand excess NMDA-receptor stimulation. In addition, studies have suggested that sodium-dependent calcium exchange can selectively protect certain cell types from excitotoxicity (Mattson et al. 1989). Other factors may include glucose oxidative potential. Glucose deprivation has been shown to potentiate excitotoxicity (Monyer and Choi 1990). Thus, a variety of factors, including neuronal EAA-receptor density, glucose oxidative potential for maintaining ion pumps, the density of other ion channels, the presence of nitric oxide synthase, and other unidentified factors, determine neuronal sensitivity to excitotoxicity.

Voltage-dependent calcium channels are likely to play a role in certain types of excitotoxicity. Studies in cultured cortical neurons have found that the dihydropyridine calcium channel antagonist nifedipine has little affect on the marked neuronal degeneration produced by brief exposure to high concentrations of NMDA. On the other hand, nifedipine markedly reduces the neurotoxicity produced by prolonged exposure to quinolinate, kainate, and AMPA (Weiss et al. 1990). Quinolinate is a weak NMDA agonist that requires prolonged exposure for neurotoxicity. Other studies in striatal neurons have shown that the dihydropyridine antagonist nitrendipine markedly reduces the rise in $[Ca^{2+}]_i$ produced by kainate (Murphy and Miller 1989). Thus, voltage-dependent calcium channels are particularly important for neurotoxicity due to non-NMDA glutamate receptors and to submaximal stimulation of NMDA receptors that require prolonged stimulation.

As mentioned above, nitric oxide may play an important role in excitotoxicity (also see Lancaster, chapter 18). Nitric oxide is formed from arginine by the action of nitric oxide synthase. In neurons, nitric oxide synthase is

calcium/calmodulin-dependent and appears to be activated by increased intracellular calcium (Bredt and Snyder 1990). Although the physiological actions of nitric oxide in the brain have not yet been clearly defined, the formation of nitric oxide is strongly linked to NMDA-receptor activation and may be involved in synaptic plasticity and long-term potentiation (Bohme et al. 1991; Schuman and Madison 1991; O'Dell et al. 1992).

NMDA-receptor activation has been shown to increase nitric oxide formation in a variety of cell culture systems via the influx of calcium through NMDA channels (Garthwaite et al. 1989). Recent evidence suggests that nitric oxide may be an important mediator of NMDA receptor-stimulated excitotoxicity. Inhibition of nitric oxide synthase activity in vitro has been reported to protect against NMDA-receptor neurotoxicity in primary neuronal cultures (Dawson et al. 1991). Further, inhibition of nitric oxide synthase in vivo dramatically reduces the volume of cortical infarcts (a measure of neuronal cell death that is strongly linked to excitotoxicity) following irreversible focal ischemia in the mouse (Nowicki et al. 1991). Thus, an important factor in excitotoxicity is likely the formation of nitric oxide following NMDA-receptor activation.

Free radical formation has been implicated in various forms of neurotoxicity (see also Hunt, chapter 15; Pellmar, chapter 16). Nitric oxide is itself a highly reactive free radical. The cytotoxic properties of nitric oxide most likely relate to its high chemical reactivity or to metabolic products of nitric oxide metabolism, such as peroxynitrate anions ($ONOO^-$) formed when nitric oxide reacts with superoxide ions.

Precedent for nitric oxide as a mediator of cytotoxicity is found in cells such as macrophages and neutrophils, which use nitric oxide as a bacteriocidal and tumoricidal agent. These cells contain a form of nitric oxide synthase that is not dependent upon calcium/calmodulin and is transcriptionally regulated. Activation of the calcium/calmodulin-dependent form of nitric oxide synthase found in neurons results in transient nitric oxide formation due to the transient nature of calcium flux. On the other hand, formation of nitric oxide following induction of the calcium/calmodulin-independent form is sustained and prolonged, resulting in a much higher concentration of nitric oxide being produced.

In the brain, it appears that at low concentrations, nitric oxide acts as a neurotransmitter or neuromodulator. However, when produced in high concentrations, such as might occur in response to prolonged or excessive stimulation of NMDA receptors following stroke or hypoxia/ischemia, nitric oxide exhibits neurotoxic actions. In addition, very recent studies have shown that brain microglia, which are frequently associated with reactivity to neuronal insult such as that from hypoxia/ischemia, also possess an inducible form of nitric oxide synthase (Zielasek et al. 1992; Chandler et al. 1992). It is not known what role, if any, formation of nitric oxide by microglia may play in the excitotoxic process. In any case, glutamate-stimulated calcium flux will clearly activate nitric oxide formation, generating

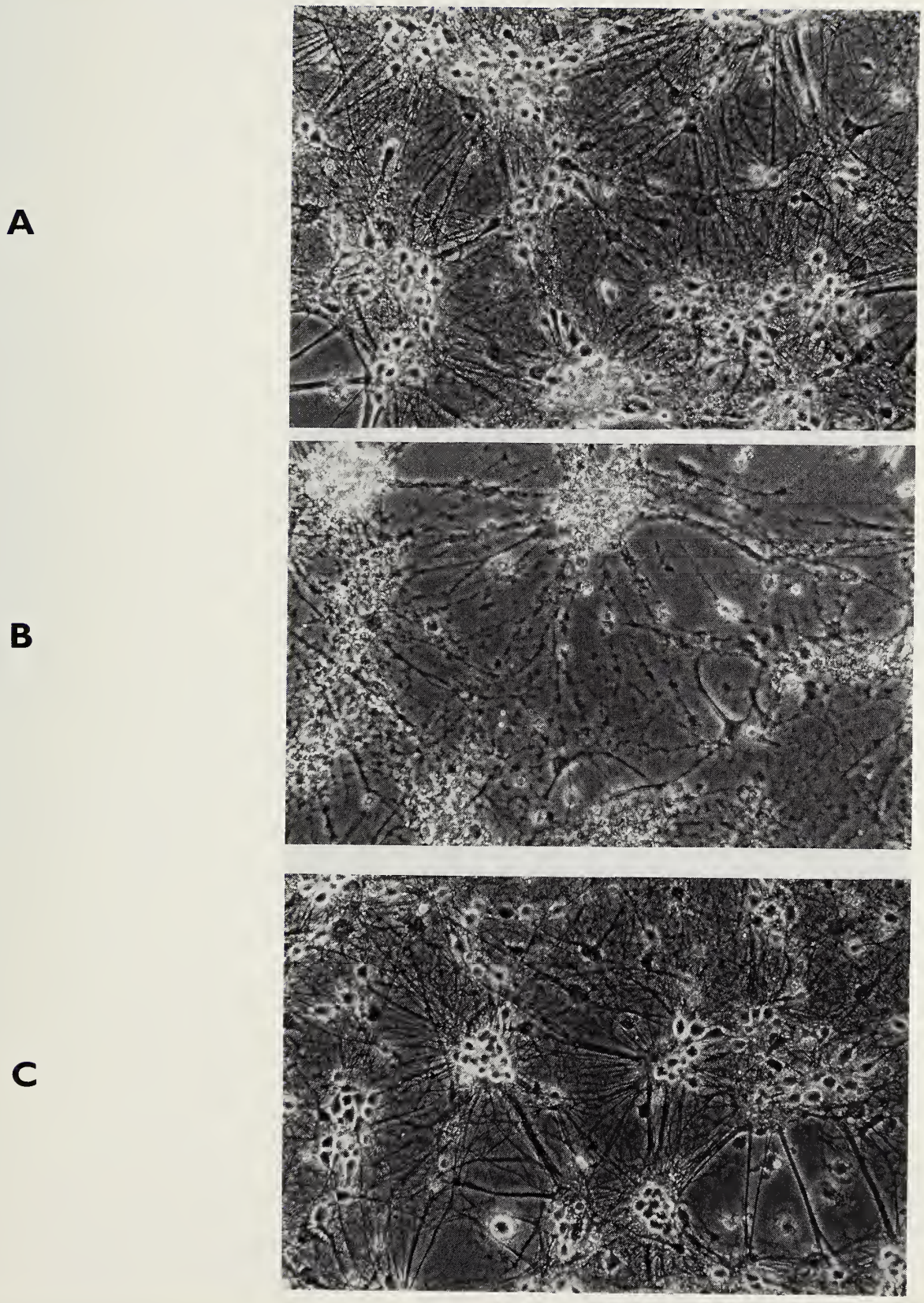

FIGURE 2

Effects of ethanol on NMDA-stimulated excitotoxicity. Shown are representative rat cortical neuronal cultures prepared as described in Chandler et al. (1992). A, Control neurons. B, Neurons treated with NMDA (100 mM) for 5 minutes and then maintained in normal media for an additional 20 hours. Note the diffuse disintegration of neuronal processes and the swollen and diffuse appearance of the cell bodies. C, Neurons treated with NMDA as described above in the presence of 100 mM ethanol for 5 minutes. Cells were then maintained in media for 20 hours just as in A and B. Note that the presence of ethanol during NMDA treatment prevents the disintegration of neuronal processes, swelling of cell bodies, and diffuse appearance of neuronal soma.

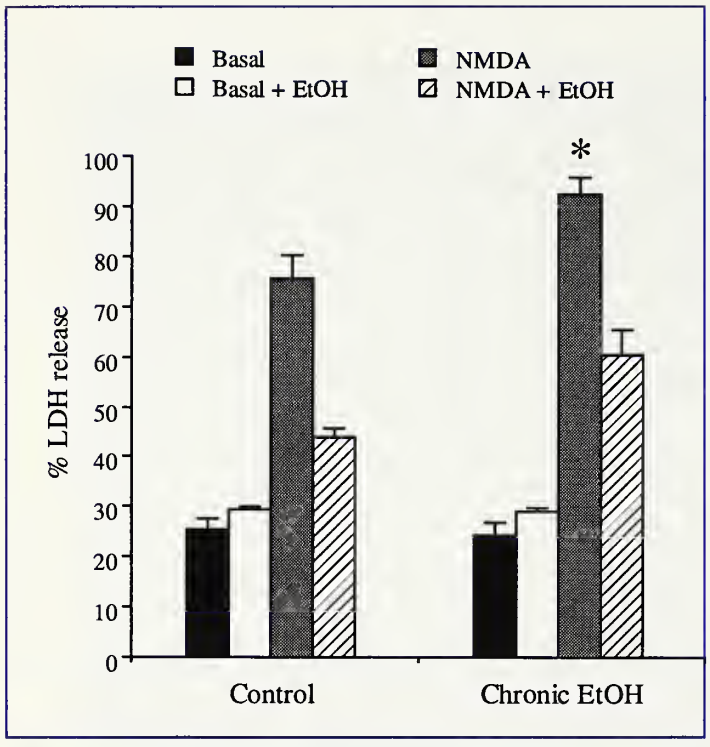

FIGURE 3

Effect of acute and chronic ethanol exposure on NMDA-stimulated neurotoxicity in cultured rat cerebral cortical neurons. Cultures were exposed to NMDA (50 μM) for 25 minutes in Mg^{2+}-free buffer; cell damage was quantitated by measuring the accumulation of lactate dehydrogenase in the culture media 20 hours after NMDA washout (Chandler et al. 1992). For determination of the effect of acute ethanol, 100 mM ethanol was added 10 minutes prior to addition of NMDA such that ethanol was present during the NMDA stimulation period but not after NMDA washout. For determination of the effects of chronic ethanol, cultures were incubated with 100 mM ethanol for 96 hours followed by removal of ethanol prior to NMDA exposure. Values represent the means ± S.E.M. of six separate experiments each performed in triplicate. Asterisk indicates significant increase ($p < 0.05$) in NMDA-stimulated lactate dehydrogenase release in cells chronically exposed to ethanol compared to nontreated controls.

free radicals and other actions that may play a key role in excitotoxicity.

ACUTE ETHANOL AND EXCITOTOXICITY

Recent studies suggest that ethanol inhibits NMDA-mediated excitotoxicity (figures 2 and 3). Exposure of cultured cerebral cortical neurons to NMDA for as little as 5 minutes results in a progressive neuronal death over the next 20 hours. Ethanol, when added during NMDA treatment, reduces the neurotoxic effects of NMDA (Chandler et al. 1992; Greenberg 1992). At maximal concentrations of NMDA, ethanol can dose-dependently block excitotoxicity, with approximately 40-percent inhibition at 25 mM ethanol and complete inhibition of excitotoxicity at 200 mM ethanol (Chandler et al. 1992). Ethanol noncompetitively antagonizes excitotoxicity by reducing the excitotoxic effects of all concentrations of NMDA that cause excitotoxicity (Greenberg et al. 1992). Thus, acute in vitro ethanol can protect neurons from EAA excitotoxicity.

Ethanol exposure alters several receptor-gated ion channels that likely underlie the protective effect of acute ethanol on excitotoxicity. A variety of biochemical and electrophysiological studies suggest that in vitro ethanol at pharmacologically relevant concentrations (e.g.,' <100 mM) is an inhibitor of NMDA receptors. Electrophysiological studies in hippocampal slices (Lovinger et al. 1990), fetal rat cerebellar Purkinje cells (Franklin and Gruol 1987), and cultured rat (Lovinger et al. 1989; White et al. 1990) and mouse hippocampal (Lima-Landman and Albuquerque 1989) neurons indicate that relatively low concentrations of ethanol (< 50 mM) inhibit NMDA-stimulated currents. Furthermore, studies in cultured cerebellar granule cells (Hoffman et al. 1989*a*,*b*) and dissociated rat brain cells (Dildy and Leslie 1989; Dildy-Mayfield and

Leslie 1991) show that low concentrations of ethanol can inhibit NMDA-stimulated calcium flux. Moreover, NMDA-stimulated norepinephrine release is inhibited by ethanol (Gonzales and Woodward 1990; Woodward and Gonzales 1990). Ethanol at higher concentrations may also inhibit other glutamate-stimulated ion channels (Gonzales and Hoffman 1991).

Besides inhibition of calcium influx through NMDA channels, ethanol inhibits calcium influx through voltage-dependent channels. Concentrations as low as 25 mM ethanol significantly inhibit fast-phase $^{45}Ca^{2+}$ influx into cerebral cortical synaptosomes (Leslie et al. 1983) and PC12 cells (Skattebol and Rabin 1987).

Electrophysiologically, ethanol reduces the calcium component of action potentials (Oakes and Pozos 1982) and inhibits calcium spikes in cultured neurons (Triestman et al. 1985). Thus, inhibition of voltage-dependent calcium channels by acute ethanol would tend to reduce $[Ca^{2+}]_i$ and excitotoxicity. Acute ethanol also enhances GABA-mediated chloride (Cl^-) flux in a certain subset of $GABA_A$ receptors (Harris and Allan 1989), which would tend to protect neurons from excessive excitation. Although these actions of ethanol would be expected to protect against excitotoxicity, there are other elements of calcium homeostasis affected by ethanol.

Ethanol can directly increase $[Ca^{2+}]_i$ by releasing intracellular stores (Daniell and Harris 1989*a*,*b*; Machu et al. 1989). Furthermore, ethanol inhibits sodium-calcium exchange in synaptic membranes (Michaelis 1989*a*). This effect may have a protective effect against further increases in excitotoxicity since reversal of sodium-calcium exchange following sodium influx may further raise intracellular calcium levels, leading to excitotoxicity. Additional studies are required to fully understand the actions of ethanol on glutamate-mediated excitotoxicity, although the inhibition of the NMDA receptor clearly represents a mechanism for ethanol's inhibition of NMDA-mediated excitotoxicity in cerebral cortical neuronal cultures.

CHRONIC ETHANOL AND EXCITOTOXICITY

Although acute ethanol treatment may protect against EAA-induced excitotoxicity, it is not clear how long this protective action observed in vitro would last. Tolerance to ethanol is remarkably rapid, occurring within hours and during a single drinking episode (Goldstein 1983). Ethanol withdrawal represents a hyperexcitable state that can include severe delirium and seizures after prolonged drinking episodes. Seizures have been associated with EAA neurotransmission and excitotoxic neuronal lesions at the site of the seizure focus. During ethanol withdrawal, neuronal hyperexcitability is likely due at least in part to increased EAA neurotransmission. NMDA antagonists reduce ethanol withdrawal symptoms (Grant et al. 1990). Further, voltage-sensitive calcium channel antagonists reduce withdrawal symptoms (Little et al. 1986). These data suggest that ethanol withdrawal hyperexcitability involves increased neuronal calcium flux that could sensitize neurons to excitotoxicity.

The loss of cortical pyramidal neurons (Harper and Kril 1990) and

decreased cognitive function are entirely consistent with chronic ethanol treatment (Oscar-Berman and Ellis 1987) sensitizing cortical neurons to excitotoxicity. Increased sensitivity to excitotoxicity during chronic ethanol administration is also consistent with both in vitro and in vivo studies examining the effects of ethanol on neurotransmission and the neuropathology of chronic ethanol abuse. Studies in cell culture have clearly indicated that chronic ethanol treatment sensitizes neurons to NMDA-mediated excitotoxicity (figure 3). Taken together, these studies are compatible with the notion that EAA excitotoxicity mediates a major component of the neurotoxic effects of ethanol.

The probable mechanism by which chronic ethanol exposure increases excitotoxicity is adaptive changes in neuronal ion channels that regulate excitability and calcium flux during chronic ethanol treatment (figure 4). Michaelis has pioneered studies for more than a decade showing increases in L-glutamate binding sites during chronic ethanol treatment (Michaelis 1989*b*; Michaelis et al. 1990, 1978). These studies have led to the cloning of the cDNA for a 70-kD glutamate binding protein that forms a heteromeric protein channel with approximately three other proteins. This channel is one form of the NMDA receptor found to play an important role in excitotoxicity (Mattson et al. 1989; Michaelis et al. 1992). Thus, the increased L-glutamate sites reported in human alcoholics and rats treated chronically with ethanol appear to represent an increase in the agonist binding site on NMDA ion channels.

Other studies have found that chronic ethanol treatment increases the density of (+)-5-methyl-10,11-dihydro-5H-dibenzo(a,b)-cyclo-hept-5,10-imine hydrogen maleate (MK-801) sites in the hippocampus (Grant et al. 1990). MK-801 is a ligand used to identify the NMDA ion channel itself. Furthermore, studies using cultured cerebellar neurons have found that chronic ethanol treatment can significantly increase NMDA-stimulated calcium influx (Iorio et al. 1992).

Increases in NMDA-receptor ion channels and NMDA-mediated calcium flux are not the only changes that would sensitize neurons to excitotoxicity. Chronic ethanol treatment of cell cultures in vitro (Brennan et al. 1989; Messing et al. 1986) and animals in vivo (Brennan et al. 1990) results in an increase in membrane calcium channels, particularly the L-type, dihydropyridine (DHP), voltage-dependent calcium channels. The L-type, voltage-gated channels are clustered at the base of major dendrites in hippocampal CA1 pyramidal neurons (Westenbroek et al. 1990), where they may play an important role in the high sensitivity of this group of neurons to excitotoxicity.

Chronic ethanol exposure also decreases GABA-stimulated chloride flux, with little change in receptor binding (Harris and Allan 1989). The decrease in GABA inhibition would reduce homeostatic mechanisms that normally prevent excessive excitation.

In addition to receptor-gated channels, chronic ethanol exposure increases the maximum velocity of the sodium-calcium antiporter (Michaelis 1989*a*). This

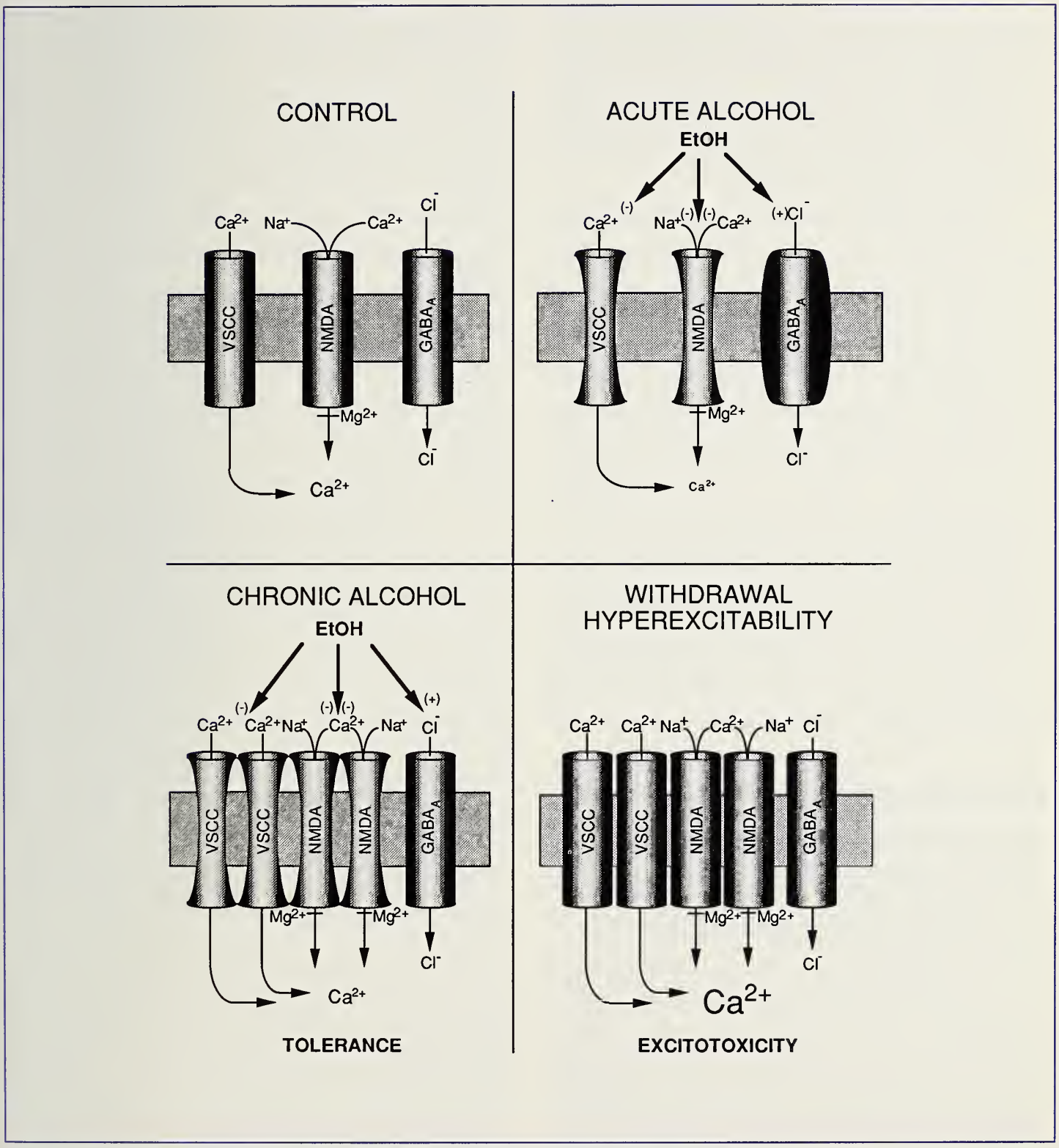

FIGURE 4

Schematic diagram of the interaction of acute and chronic ethanol with particular ion channels. Under control conditions, neuronal excitability is enhanced by NMDA receptor-mediated and voltage-sensitive calcium channel-mediated ion flux. The $GABA_A$ channel reduces neuronal excitability by stimulating Cl^- efflux, which tends to hyperpolarize neurons. In the presence of acute ethanol, neuronal excitation and $[Ca^{2+}]_i$ is blunted due to enhanced $GABA_A$ flux and decreased NMDA and voltage-sensitive calcium channel activity. During *chronic ethanol*, adaptive mechanisms increase NMDA and voltage-sensitive calcium channel density, and the $GABA_A$ channels lose their responsiveness to ethanol. This adaptive change may represent one component of tolerance to ethanol in which $[Ca^{2+}]_i$ returns to levels comparable to that found in controls. During withdrawal from ethanol, the increased calcium flux secondary to the presence of more NMDA and voltage-sensitive calcium channels causes hyperexcitability often associated with ethanol withdrawal and sensitizes neurons to excitotoxicity by increasing $[Ca^{2+}]_i$. This sensitization to excitotoxicity may underlie the neurodegeneration associated with chronic alcohol abuse.

change in antiporter might protect, or it could play a role in excitotoxic increases in calcium, as the influx in sodium might actually reverse the antiporter, leading to further increases in $[Ca^{2+}]_i$.

Furthermore, recent studies suggest that chronic ethanol increases receptor-stimulated production of nitric oxide (Davda et al., in press). Although these studies were not done in neurons, an increase in NMDA-stimulated nitric oxide production would be expected to increase excitotoxicity. However, ethanol does not change the sensitivity of neurons to nitric oxide-mediated toxicity (Greenberg et al. 1992). Thus, a variety of data suggest that chronic ethanol treatment may disrupt calcium homeostatic mechanisms and enhance EAA excitotoxicity.

SIMILARITIES AND INTERACTIONS OF ETHANOL NEUROPATHOLOGY WITH ISCHEMIC BRAIN DISEASE AND TRAUMATIC BRAIN INJURY

Several studies have shown that chronic alcoholics (Carlen et al. 1978; Harper et al. 1985, 1987) and even heavy social drinkers (Cala et al. 1983) show cerebral atrophy with dilatation of the ventricles and cerebellar vermal atrophy (Pfefferbaum et al. 1990; see also Pfefferbaum and Rosenbloom, chapter 4). The gross brain histopathology of alcoholics is characterized by a uniformly diffuse atrophy of the cerebral cortex (Freund 1985; see also Harper and Kril, chapter 3). Microscopic changes consist of a diffuse and patchy loss of neurons with dendritic and axonal regression in many remaining neurons. Cerebral shrinkage is largely explained by a loss of cortical white matter, which results from a loss of myelinated fibers projecting from or to the cerebral cortex (Harper et al. 1989). These neuronal changes are not specific for alcohol abuse and are not distinguishable from those that occur during aging and/or from hypoxia (Lynch 1960). Furthermore, ethanol intoxication frequently precedes symptoms of ischemic brain damage, particularly among patients under 40 years of age (Hillbom 1978). Hypoxic-ischemic brain damage is strongly linked to EAA-mediated excitotoxicity (Choi 1990; Clark 1989). Although alcohol-associated atrophy in the central nervous system is well-documented in humans, these findings are complicated by problems of nutrition. However, Walker's studies with rats receiving nutritionally complete diets have shown definitive evidence that chronic ethanol consumption has neurotoxic effects (Walker et al., chapter 11).

Chronic ethanol abuse produces a significant loss of hippocampal CA1 pyramidal cells, dentate granule cells, and interneurons. Furthermore, the surviving neurons have attenuated dendrites and decreased density of dendritic spines. These changes are associated with memory deficits in animals comparable to the dementia often found in alcoholics (Walker and Hunter 1987; Walker et al. 1981).

Although ethanol is neurotoxic, the exact mechanisms are not known. The neuropathological similarities of chronic ischemia and ethanol abuse may be due to overlapping pathological etiologies. Ischemic brain disease and stroke are asso-

ciated with chronic alcohol abuse. Both the World Health Organization and the Stroke Council list chronic ethanol abuse as a risk factor for stroke (Gorelick 1990). Epidemiologic studies have associated ethanol consumption with strokes, particularly ischemic stroke; more than 3 drinks per day has been suggested as a threshold level (Gorelick 1990). Cerebral ischemia is the largest category of stroke. Ischemia has been shown to increase extracellular levels of glutamate more than tenfold (Clark 1989). By releasing more glutamate, this process secondarily excites neurons.

The excitotoxic hypothesis of brain injury proposes that glutamate is a principal cause of damage in ischemia. Three components of this hypothesis have been tested and largely proven in experimental studies. First, elevated concentrations of glutamate cause excessive excitation at a subset of glutamate receptors, the NMDA receptor. Second, excitation at this receptor leads to excessive influx of sodium, chloride, and water, causing acute neuronal damage, and calcium, causing delayed and more permanent damage. Third, pharmacologic blockade at the NMDA receptor-ion channel complex prevents ischemic neuronal damage (Choi 1990; Clark 1989). Ethanol could sensitize neurons to neuronal damage due to increases in NMDA- and voltage-dependent calcium channel flux, reductions in GABA feedback inhibition, and other effects. The increased susceptibility of neurons to cell death following ethanol exposure could represent a major component of the neurotoxic effects of ethanol.

CONCLUSIONS

Ethanol has a distinct interaction with several elements related to excitotoxicity. Excitotoxicity occurs through the activation of a heterogeneous group of EAA receptors that are likely to have a differential sensitivity to ethanol. The NMDA family of EAA receptors is the most excitotoxic and the most sensitive to acute inhibition by ethanol. This inhibition by ethanol probably contributes to the sedative and cognitive disrupting effects of ethanol.

During chronic ethanol administration, adaptive changes involving tolerance to ethanol result in increases in NMDA and voltage-sensitive calcium channel density that contribute to the sensitization of neurons to excitotoxicity. During ethanol withdrawal hyperexcitability, it is likely that certain neurons that have undergone adaptive increases in calcium channels reach a threshold of excitation that triggers neuronal death. The similarity of the neuropathology of hypoxic-ischemic brain damage and chronic ethanol abuse is consistent with excitotoxic neuronal death occurring over a prolonged period, leading to diffuse neuronal loss and decreases in brain function and size.

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18

NITRIC OXIDE AND ETHANOL-INDUCED BRAIN DAMAGE—A HYPOTHESIS

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INTRODUCTION

Nitric oxide is a novel neuronal messenger (Bredt and Snyder 1992) that may represent a new class of neurotransmitters (Snyder 1992). Evidence also suggests that nitric oxide may mediate N-methyl-D-aspartate (NMDA)-induced excitotoxicity (see Crews and Chandler, chapter 17), a process associated with chronic ethanol exposure. Although little research has been reported implicating nitric acid in ethanol-induced neurotoxicity, basic research on nitric oxide and its importance to several physiological and pathological processes is expanding rapidly. Thus, this chapter is included to introduce the reader to the possible role of nitric acid in ethanol-induced brain damage. The initial sections of this chapter provide background material to familiarize the reader with nitric oxide.

CHARACTERISTICS AND ACTIONS OF NITRIC OXIDE IN THE BRAIN

Nitric oxide, a small molecule, is a simple gas with free radical properties. From the original characterization of nitric oxide as a "cell killer" of white blood cells, rapid advances in research have enlarged the known functions of nitric oxide. These functions include mediating (1) endothelial-derived relaxing factor (EDRF), which is responsible for the vasodilation triggered by acetylcholine and bradykinin; (2) nonadrenergic/noncholinergic (NANC) neuroenteric activity, which allows relaxation of the gut to receive food; (3) neurotransmission by neurons innervating the corpus cavernosum and controlling erection (Rajfer et al. 1992; Burnett et al. 1992); (4) neurotransmission by neurons innervating cerebral arteries (Snyder 1992); and (5) neurotransmission in the brain (Bredt et al. 1990). As researchers have explored the actions of nitric oxide in the brain, additional functions have been predicted for this molecule in the nervous system (Lancaster 1992*b*), a number of which may be involved in the actions of ethanol on the brain.

Location of Nitric Oxide in the Brain

Since nitric oxide is an extremely labile compound, localization of nitric oxide in

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neurons is dependent on techniques capable of locating the synthetic enzyme nitric oxide synthase. All known types of nitric oxide synthase transform L-arginine into nitric oxide and L-citrulline, requiring nicotinamide adenine dinucleotide phosphate (NADPH) as an electron donor (Bredt and Snyder 1992). Conveniently, nitric oxide synthase-containing neurons can be identified with NADPH-diaphorase staining (Dawson et al. 1991; Bredt et al. 1991), because nitric oxide synthase and NADPH diaphorase are co-localized throughout the peripheral and central nervous systems. Newer methods including immunohistochemistry (Bredt et al. 1990) and in situ hybridization (Bredt et al. 1991) confirm earlier reports from the diaphorase studies. However, immunohistochemical techniques identify only EDRF and neuronal nitric oxide synthase. Consequently, the identification and localization of microglial and macrophage nitric oxide synthase are currently unknown. Some uncertainty exists regarding the type of nitric oxide synthase in astroglia. Although Murphy et al. (1990) reported nitric oxide synthase activity in cultured astrocytes, staining for nitric oxide synthase is negative in glial cells in the brain.

The heaviest concentration of nitric oxide synthase staining in the brain is in the cerebellum, olfactory bulb, and accessory olfactory bulb. Within the cerebellum, the highest concentration of nitric oxide synthase is found in the granule cell layer (Southam et al. 1992). Nitric oxide synthase is also found in the molecular layer as well as in the processes of granule cells projecting to this area. Basket cells have a high concentration of nitric oxide synthase, whereas Purkinje cells contain none.

When nitric oxide is released, it diffuses quickly to cells and acts on second messenger systems. In Purkinje cells, nitric oxide binds to the iron in guanylyl cyclase and activates the enzyme to produce cyclic GMP (cGMP), which in turn regulates protein kinases, phosphodiesterases, and ion channels (Vincent and Hope 1992). Stimulation of NMDA receptors also increases the influxes of sodium, chloride, water, and calcium. Activation of G-proteins triggers the generation of neuronal messengers, such as inositol-1,4,5-trisphosphate (IP_3) (Ferris and Snyder 1992), which in turn release calcium from intracellular stores. Although some controversy exists (Linden and Connor 1992), it has been suggested that nitric oxide in the cerebellum is also involved in producing long-term depression (LTD), a process required for cerebellar motor learning (Shibuki and Okada 1991).

Glial cells are likely involved in nitric oxide reactions, since cGMP is found in astrocytes of the cerebellar granule cell layer and in Bergmann glial cell processes radiating through the molecular layer (de Vente et al. 1989). Also, glial cells may participate in regulating nitric oxide production by supplying arginine to the neurons for the reaction (Aoki et al. 1991). Astrocytes in culture release nitric oxide in response to α_1-adrenergic receptor stimulation by epinephrine and quisqualate receptor stimulation by glutamate (Murphy et al. 1990; Agullo and Garcia 1992). On the other hand, nitric oxide is

released from neurons primarily in response to glutamate stimulation of NMDA receptors.

Recent extensive mapping of nitric oxide synthase in rat brain (Vincent and Kimura 1992) shows heavy staining of selected cells within the cortex, hippocampus, and striatum, although staining overall is scattered. NADPH-diaphorase staining throughout the visual pathway from receptor to cortex illustrates the importance of nitric oxide in vision (Provis and Mitrofanis 1990). The presence of NADPH-diaphorase neurons in the cortex (Thomas and Pearse 1964) is well documented by histochemistry and by antibodies against nitric oxide synthase. These cells appear resistant to neurotoxicity resulting from excessive stimulation of NMDA receptors, similar to other neurons that contain nitric oxide synthase and produce nitric oxide (Koh and Choi 1988).

A subpopulation of basal forebrain cholinergic neurons exhibits the NADPH-diaphorase-reaction and may also contain galanin, a peptide that inhibits the actions of acetylcholine. Nitric oxide may be produced in this brain area and may be involved in regulating blood flow within the cerebral cortex (Arneric et al. 1990). Perturbations of cerebral blood flow by ethanol may involve nitric oxide.

Nitric oxide synthase-containing neurons in the septum have projections to the hippocampus (Kinjo et al. 1989), and scattered cells in the dentate hilus and molecular layer stain for nitric oxide synthase. CA1 pyramidal cells and the granule cells of the dentate gyrus do not stain for nitric oxide synthase (Vincent and Kimura 1992), although nitric oxide generation in the hippocampus in response to NMDA activation has been reported (East and Garthwaite 1991). These results suggest that CA1 pyramidal cells may contain a different isoform of nitric oxide synthase (Bredt et al. 1991), because nitric oxide, acting as a putative retrograde factor in long-term potentiation (LTP), enhances spontaneous neurotransmitter release from hippocampal brain slices (Schuman and Madison 1991). Hippocampal function and LTP are known to be sensitive to ethanol exposure.

NADPH-diaphorase-positive cells in the striatum also stain positively for neuropeptide Y and somatostatin. Although these cells are sensitive to kainic acid-induced toxicity, significant resistance to NMDA-induced toxicity is observed in these cells. Interestingly, these cells are selectively spared in Huntington's disease (Ferrante et al. 1987) and are resistant to a variety of toxins (Koh et al. 1986; Koh and Choi 1988). The mechanism responsible for protection of these neurons remains unclear. One hypothesis suggests a superior ability to resist free radical damage (Vincent and Kimura 1992), perhaps through a diaphorase mechanism (Murphy et al. 1990). Others propose that nitric oxide can reduce toxic intracellular calcium levels unrelated to cGMP (Garg and Hassid 1991). Protection or involvement of these cells in ethanol-induced brain damage has not been investigated.

Nitric oxide synthase has been detected in the paraventricular and supraoptic nuclei of the hypothalamus (Bredt et al.

1990), indicating a potential role for nitric oxide in posterior pituitary regulation. Interestingly, both nitric oxide and arginine vasopressin (AVP) are concentrated together in neurons and have L-arginine as a common precursor. Thus, it is possible that nitric oxide may be linked to AVP activity in the development of tolerance to ethanol.

Nitric oxide synthase staining is also found in a population of mesopontine cholinergic neurons. These neurons are thought to regulate the thalamus through the activation of nitric oxide and atriopeptides on guanylyl cyclase to produce cGMP. Disease conditions associated with the degeneration of these neurons include supranuclear palsy and idiopathic Parkinson's disease (Hirsch et al. 1987). The role of ethanol and nitric oxide in the etiology and progression of these degenerative disorders is unknown.

Targets of Nitric Oxide Action in Brain

In the cerebellum, no simple anatomical pathway is available to explain which cells generate nitric oxide and which cells generate cGMP in response to nitric oxide (Southam et al. 1992). Some of the cells in the cerebellum do not participate in this interaction, whereas granule cells can produce both nitric oxide and cGMP, but apparently not simultaneously. It appears that calcium ions entering the neuron and stimulating nitric oxide synthase to produce nitric oxide also inhibit cGMP synthesis (de Vente et al. 1989). Cerebellar glial cells, including Bergmann glia and astrocytes, are also targets for nitric oxide and produce cGMP in response. Thus, nitric oxide is hypothesized to act as a neurotransmitter that moves from presynaptic to postsynaptic areas, as a retrograde messenger that moves from postsynaptic to presynaptic areas, and as a messenger between neurons and glial cells.

Stimulation of Nitric Oxide Formation

Nitric oxide is synthesized by macrophages, neutrophils, endothelial cells, Kupffer cells and hepatocytes, kidney epithelial cells, the adrenal gland, NANC nerves, microglia, and neurons. Stimulation for nitric oxide synthesis is dependent on cellular location (Salter et al. 1991) and type of nitric oxide synthase contained in the generator cell. At least three types of nitric oxide synthase are currently known and others have been proposed, including one which requires catalase. These enzymes include the nitric oxide synthase found in macrophages and neutrophils and another found in neurons and endothelial cells.

Nitric oxide formation is triggered by several events depending on whether the nitric oxide synthase involved is the inducible form (synthesized by specific molecular signal) found in macrophages, neutrophils, hepatocytes, smooth muscle cells, fibroblasts, mesangial cells, and some tumor cells or the constitutive form (always present) found in neurons and endothelial cells. In neurons and endothelium, elevated intracellular free calcium ions are sufficient to trigger enzymatic activity, which is also dependent on calmodulin. A similar enzyme is found in platelets, adrenal gland, and lung (Klatt et al. 1992). Brain nitric oxide synthase is

regulated by calcium calmodulin kinase II (Bredt et al. 1992), which phosphorylates the enzyme and reduces activity, and by protein kinase C, which phosphorylates and activates the enzyme (Nakane et al. 1991).

ROLE OF NITRIC OXIDE IN PHYSIOLOGICAL AND PATHOLOGICAL PROCESSES

Nitric Oxide and Excitotoxicity

Excessive amounts of glutamate are known to exert a toxic effect on neurons containing glutamate receptors through excitotoxicity, a process in which nitric oxide (Dawson et al. 1991) and increased cGMP levels participate (Frandsen et al. 1992). Although excessive levels of glutamate affect all glutamate receptors, the neurotoxicity associated with cerebral ischemia (Choi 1991) primarily involves NMDA receptors. With excessive stimulation of NMDA receptors, intracellular calcium levels rise, resulting in cell death. Nitric oxide also binds to the iron portion of enzymes and increases production of cGMP in neurons and glia, altering metabolism in the affected cells. Inhibitors of nitric oxide synthesis prevent NMDA excitotoxicity, whereas administration of L-arginine reverses the inhibition and increases excitotoxicity (Dawson et al. 1991). These results implicate nitric oxide in the neurotoxicity of excessive glutamate stimulation (Bredt and Snyder 1992).

The cellular sources of nitric oxide associated with excitotoxicity most likely include nitric oxide synthase-containing neurons and microglia. Astrocytes may be involved in the process of excitotoxicity, considering evidence that astrocytes are involved in removal of glutamate from synaptic areas and metabolism of glutamate (Teichberg 1991), and considering evidence that astrocytes and neurons are linked through nitric oxide action as a messenger molecule (Ishizaki et al. 1991).

Ethanol, Nitric Oxide, and Excitotoxicity

Both nitric oxide (Bredt et al. 1990; Ross et al. 1990) and ethanol have been linked to brain damage associated with excitotoxicity (Chandler et al. 1991; Lustig et al. 1992*a*; see also Crews and Chandler, chapter 17). Cultured cortical neurons respond to NMDA with excitotoxicity and cell death through elevation of free intracellular calcium levels and activation of calcium-dependent reactions (Lustig et al. 1992*a*). NMDA toxicity can be blocked by NMDA-receptor antagonists and by nitric oxide synthesis inhibitors. Acute ethanol exposure of cortical neurons in culture inhibits NMDA excitotoxicity (Takadera et al. 1990; Chandler et al. 1991; Lustig et al. 1992*a*), suggesting that ethanol might inhibit toxicity through interference with the action of nitric oxide (see also Crews and Chandler, chapter 17). However, acute ethanol exposure of cortical cells in culture apparently protects the cells against NMDA-mediated toxicity, but not against sodium nitroprusside toxicity (Lustig et al. in press). These results suggest that the inhibitory action of acute ethanol exposure on excitotoxicity is mediated through interven-

tion early in the process of NMDA receptor stimulation, rather than through direct inhibition of nitric oxide.

Although acute ethanol exposure inhibits glutamate stimulation of NMDA receptors, and thus inhibits excitotoxicity, chronic ethanol exposure leads to increased sensitivity of ion channels and upregulation of NMDA receptors, which contribute to excitotoxicity. These changes have been observed in studies of the brains of alcoholics (Michaelis et al. 1990), neurons in culture (Messing et al. 1990), and in vivo experiments with animals (Brennan et al. 1990). Since nitric oxide powers excitotoxicity (Dawson et al. 1991), it is possible that chronic ethanol abuse through stimulation of excitotoxicity leads to excessive production of nitric oxide, followed by brain damage. Recent information indicates that excitotoxic injury mediated by nitric oxide in cerebral cortical cultures does not require the action of cGMP. This finding suggests that the direct toxic actions of nitric oxide and its reactive metabolites are responsible for cell death (Lustig et al. 1992*b*).

Free Radical Properties of Nitric Oxide

Nitric oxide damages cells, not only through its own free radical properties, but through formation of additional free radicals (Beckman et al. 1990; Snyder 1992). Nitric oxide also exerts damage through ADP-ribosylation (Brune and Lapetina 1989) and DNA deamination and genotoxicity (Wink et al. 1991). Nitric oxide reacts with superoxide free radical to form peroxynitrite (Beckman et al. 1990). Peroxynitrite, in turn, forms hydroxide free radical and NO_2 free radical, which are more toxic to cells than nitric oxide. Brain damage following cerebral ischemia may be mediated by the combination of nitric oxide produced through excessive glutamate stimulation of NMDA receptors, followed by binding to oxygen during reperfusion of the ischemic tissues. During this postischemic period, extensive brain damage may be mediated by hydroxyl radicals formed from peroxynitrite, a reaction inhibited by blockers of nitric oxide synthesis and restimulated by L-arginine (Nowicki et al. 1991). An action of ethanol through this free radical mechanism has not been reported.

Ethanol, Nitric Oxide, and Seizures

Ethanol-related withdrawal seizure activity may lead to brain damage. Nitric oxide production related to NMDA-receptor stimulation may be responsible for the seizure activity and excitotoxicity observed in some individuals during ethanol withdrawal (Gulya et al. 1991; Grant et al. 1990; Hoffman et al. 1990). Thus, glutamate excitotoxicity mediated by nitric oxide may play an important role in generating the neurotoxicity associated with ethanol withdrawal related to chronic ethanol abuse (see Lancaster 1992*a* for review).

Ethanol, Free Radicals, and Nitric Oxide

In pathological conditions, including chronic ethanol abuse, the production of nitric oxide, superoxide, and subsequent hydroxyl radical formation may be increased sufficiently to modify proteins

and to contribute to lipid peroxidation (Hogg et al. 1992). Although ethanol can act as a free radical scavenger, in conditions associated with chronic ethanol abuse, free radical production may be increased (see Hunt, chapter 15; Pellmar, chapter 16) and natural protection from oxidative damage may be compromised (Boveris et al. 1983; Klein et al. 1983). Protective systems including superoxide dismutase (Vincent and Hope 1992) and glutathione are decreased in chronic ethanol abuse, leading to vulnerability to tissue damage. It appears that certain individuals are more sensitive to brain damage associated with chronic ethanol abuse, indicating the potential involvement of both genetic and environmental factors.

Nitric Oxide and LTP

LTP at glutamate synapses in the hippocampus is used as a model for learning and memory. The proposed model suggests that glutamate, through stimulation of NMDA receptors on the postsynaptic cell, increases calcium influx, stimulating a calmodulin-dependent nitric oxide synthase to produce nitric oxide. Nitric oxide diffuses back to the presynaptic terminal, stimulating guanylyl cyclase to produce cGMP, which in turn leads to production of more glutamate (figure 1).

Since the postsynaptic cell appears to persistently stimulate the presynaptic cell in response to glutamate release, the LTP model of learning and memory requires the presence of a postsynaptic to presynaptic retrograde messenger. Because

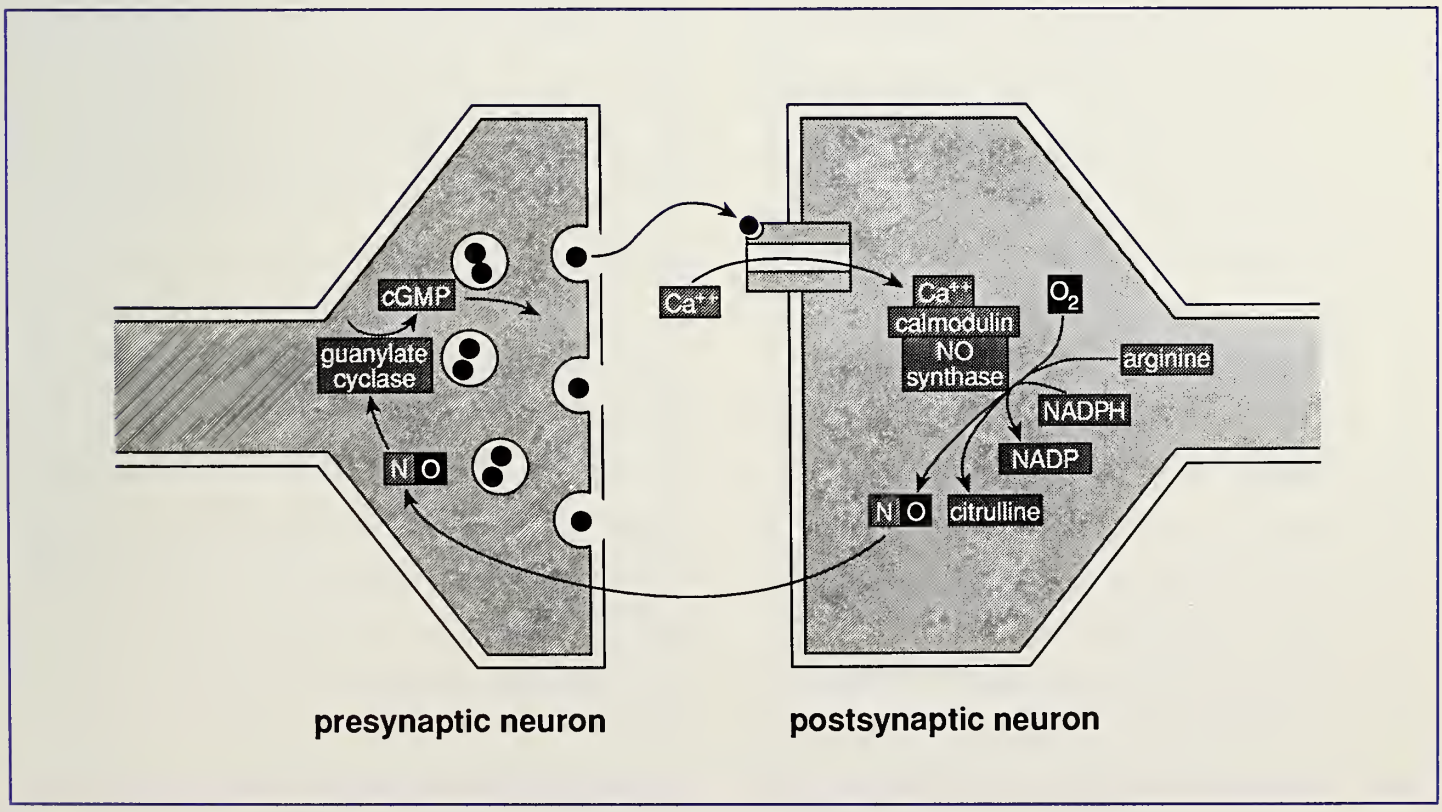

FIGURE 1

Glutamate, through stimulation of NMDA receptors on the postsynaptic cell, increases calcium influx, stimulating a calmodulin-dependent nitric oxide synthase to produce nitric oxide. Nitric oxide diffuses back to the presynaptic terminal, stimulating guanylyl cyclase to produce cGMP, which in turn leads to production of more glutamate.

antagonists of nitric oxide synthesis block LTP in hippocampal slices, researchers speculate that nitric oxide is the retrograde messenger in LTP expression (Schuman and Madison 1991). One problem with this proposal is that the postsynaptic pyramidal cells in the hippocampus, which are proposed to produce nitric oxide, are unresponsive to antibodies or stains for known types of nitric oxide synthase. These results suggest that if indeed nitric oxide is the retrograde messenger, a presently unidentified type of nitric oxide synthase may be active in pyramidal cells (Snyder 1992).

Ethanol, Nitric Oxide, and LTP

The memory impairing effects of acute and chronic ethanol use are well known. Apparently, nitric oxide and LTP are involved in several types of memory and learning deficits associated with ethanol use (for reviews of ethanol and LTP, see Hunter, chapter 13; Pellmar, chapter 16). The mechanism for memory impairment associated with intoxication (Sinclair and Lo 1986) may involve interference with nitric oxide, the proposed retrograde messenger for LTP (Schuman and Madison 1991). Even mild intoxication may interfere with memory based on LTP expression. For example, ethanol concentrations in rat hippocampus in concentrations as low as 5 mM, an amount equivalent to that achieved following one drink, depress LTP (Blitzer et al. 1990). Further research is needed to determine the role of nitric oxide in LTP expression following chronic ethanol abuse, considering the many problems regarding cognitive function and learning and memory in affected individuals.

Nitric Oxide and Immune Responses in the Brain

In macrophages and other inducible cells, nitric oxide synthase is calcium-independent but can be induced by cytokines including γ-interferon and interleukin-1-β. Inducible cells begin nitric oxide formation several hours after exposure to cytokines and microbial products and continue releasing nitric oxide for many hours without calcium or calmodulin requirements. Recent information suggests that inducible nitric oxide synthase is activated by immunologic activity at the transcriptional level after the cells are exposed to γ-interferon and lipopolysaccharide (Xie et al. 1992). Neutrophilic nitric oxide synthase also responds to these immunologic factors, requires calcium ions for initiation of enzyme activity, but is insensitive to calmodulin (Klatt et al. 1992).

Inducible and constitutive nitric oxide synthases share only 51 percent of their amino acid sequences, allowing identification in cells through polyclonal antibody testing. Both enzymes contain noncovalently bound flavin adenine dinucleotide and flavin mononucleotide but differ in amino acid sequences related to calmodulin binding. Nitric oxide synthase enzyme activity of both types is similar to cytochrome-P450 reductase.

The type of nitric oxide synthase in microglia is unknown, but since the enzyme is inducible, it is thought to be the macrophage type. Microglia, and astro-

cytes to a fractional degree, are induced to produce nitrite, a product of the nitric oxide pathway, in response to γ-interferon and bacterial lipopolysaccharide (Zielasek et al. 1992).

Lipopolysaccharide enhances the formation of nitric oxide from endogenous L-arginine in cultured astrocytoma cells (Salvemini et al. 1992) and induces nitric oxide synthase activity in microglia, primary astrocyte cultures, and C6 glioma cells, but not in neuron-like, N18-neuroblastoma cells (Simmons and Murphy 1992). Electrical stimulation of the white matter in rat cerebellar slices results in the release of L-arginine. Since L-arginine is the natural precursor of nitric oxide, these results suggest that neuronal stimulation of glial cells may result in release of arginine to supply nitric oxide synthase (Hansel et al. 1992).

Autoreactive T lymphocytes secreting γ-interferon may activate microglia and brain macrophages to secrete nitric oxide, which in turn may injure myelin or brain parenchyma. Cytotoxic action of microglia against oligodendroglia (Merrill and Zimmerman 1991) and autoimmune destruction of islet cells by nitric oxide (Kroncke et al. 1991) support a potential role of nitric oxide in autoimmune inflammatory and demyelinating nervous system diseases (see Lancaster, chapter 19).

Thus, nitric oxide is used by macrophages, microglia, and other cells of the immune system to kill foreign organisms, and to modulate inflammatory and immune responses against endotoxins and cellular components deemed foreign (Collier and Vallance 1989). Sometimes, nitric oxide actually decreases the inflammatory response (Kawabe et al. 1992), perhaps as a negative feedback. For example, nitric oxide produced by neutrophils and acting in conjunction with prostaglandins (Boeynaems and Pearson 1990) prevents platelet aggregation, limiting clot formation to the area of endothelial damage. Nitric oxide produced by endothelial cells inhibits neutrophil aggregation and adhesion to vascular walls (Kubes et al. 1991), affording protection against ischemia-reperfusion inflammatory injury. Recent information indicates that macrophage production of nitric oxide serves as a negative feedback signal to inhibit lymphocyte proliferation (Denham and Rowland 1992; Fu and Blankenhorn 1992).

Ethanol, Nitric Oxide, and Immune Responses

Additional factors that may contribute to brain damage involving nitric oxide and ethanol include the strength of the immune response elicited in brain macrophages and microglia. In response to autoimmune sensitization and enhanced antigen presentation, which may occur following tissue damage associated with chronic ethanol abuse (see Lancaster, chapter 19), excessive amounts of nitric oxide may be released by microglia and macrophages. This response would lead to more tissue damage. If nitric oxide suppresses proliferation of suppressor T cells, incidence of autoimmunity could be increased as is observed in chronic alcoholics.

In some individuals, progressive brain damage, particularly in the white matter, continues following the removal of ethanol. Perhaps autosensitization against neuronal and glial antigens continues to stimulate nitric oxide release from microglia and macrophages, promoting autoimmune inflammatory disease (Kroncke et al. 1991) even in the absence of ethanol.

SUMMARY AND CONCLUSIONS

Nitric oxide potentially contributes to ethanol-related brain damage in a variety of ways. Not only does nitric oxide participate in neuronal function as a neurotransmitter and glial messenger, but also as an immune factor and cerebral blood flow modulator. If ethanol alters normal levels of nitric oxide, either directly or indirectly through free radical mechanisms, excitotoxicity, or immune reactions, it is possible that an array of functions that are modulated by nitric oxide could be influenced by ethanol intake. Like ethanol, nitric oxide affects diverse populations of cells and, like ethanol, the effects of nitric oxide may depend partially on the concentrations to which the cells are exposed. Particularly in the role of excitotoxicity, ethanol and nitric oxide induce neuronal injury in association with an NMDA response.

Ethanol and nitric oxide may be involved in direct neuronal injury, microglia/macrophage-mediated neuronal injury, and astrocyte-mediated neuronal injury (Zielasek et al. 1992). Recent information (Lipton 1992) implicating NMDA-induced neuronal and astrocyte injury in HIV-associated brain damage raises the possibility that ethanol abuse may indeed have a role in the progress of neurotoxicity (Zielasek et al. 1992) of patients with AIDS dementia and encephalopathy (Lipton 1992). Thus, new research to understand the intricate dependency of neuronal and glial cell networks should provide an interesting forum to study the interactions of ethanol and nitric oxide in the nervous system.

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19

ETHANOL AND WHITE MATTER DAMAGE IN THE BRAIN

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INTRODUCTION

In previous chapters, considerable discussion has addressed extensive changes in white matter that can occur after long-term consumption of ethanol (Charness, chapter 2; Harper and Kril, chapter 3). In this chapter, these effects will be examined from a different perspective. A hypothesis will be presented suggesting that the changes in white matter might be related to autoimmune reactions analogous to those involved in multiple sclerosis (MS) and other diseases characterized by white matter damage in the brain. The discussion will briefly explore actions of ethanol on the structure and chemistry of myelin in humans and animals after ethanol ingestion and examine the role of alterations in glial cells in the progression of these effects. Finally, the hypothesis will be offered to provide a mechanism for the damage to myelin induced by ethanol exposure.

CENTRAL NERVOUS SYSTEM CHANGES IN HUMANS

For many years, clinicians and pathologists have recognized the clinical signs and subsequent pathology of white matter damage in severe alcoholics (Harper and Kril, chapter 3). Pathology of the white matter (de la Monte 1988) is most often expressed as plaque formation along with ventricular dilatation and brain shrinkage in the brains of alcoholics who expired due to accidents or from the consequences of many years of heavy drinking. The relevance of these findings to the general population of drinkers is unclear, however, because these studies have been limited largely to male alcoholics who were housed in government hospitals before death, or who were "skid-row" alcoholics. Furthermore, determining the cause of brain damage in these cases has been clouded by associated malnutrition and consequent vitamin deficiencies, which are known to alter nervous system function (Victor and Laureno 1978).

Brain damage associated with ethanol use is apparently more common than previously expected (Lishman et al. 1987). Scanning technology has revealed alterations in the brains of asymptomatic drinkers (Gallucci et al. 1989), which warns that superficial clinical examination cannot be relied upon for detection of brain damage.

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In computerized tomography (CT) and magnetic resonance imaging (MRI) scans, white matter damage appears diffusely, but not uniformly, throughout the brains of alcoholics (see Pfefferbaum and Rosenbloom, chapter 4). Interestingly, there are distinct differences in the incidence and progression of both liver (Jones and Jones 1980) and brain damage (Jacobson 1986) of males compared to females. Females appear to be at greater risk than males at an earlier stage in their drinking history, although the reason for this accelerated risk is not yet well defined (see Glenn, chapter 9).

Brain damage in alcoholics has been frequently attributed to malnutrition. However, the role of nutrition and alcoholic brain damage in the adult remains unclear. Brain scans of large populations have reported a high incidence of cortical shrinkage and ventricular dilatation in individuals without overt signs of malnutrition. Thus, some controversy remains regarding the role of ethanol, rather than malnutrition, in brain damage associated with ethanol abuse.

With advancing age, there is increased risk for brain damage due to ethanol abuse (Lishman et al. 1987), with late onset drinkers affected as severely as those who began drinking while young. History of heavy ethanol use is associated with a relative increase in total protein in white matter, increased water content of white matter, and decreased RNA in gray matter. These changes accompany acceleration of age-related loss of myelin (Wiggins et al. 1988).

Harper and Kril (1990, chapter 3) reported that changes in white matter are responsible for the brain shrinkage, ventricular dilatation, and increased cerebrospinal fluid (CSF) volume associated with chronic ethanol use. Although some reversibility of these changes (Artmann 1981; Carlen et al. 1978; Carlen and Wilkinson 1987*a*; Cala 1982, 1987) may be observed with abstinence (Muuronen et al. 1989), in many individuals some degree of ventricular dilatation and white matter damage persist. In some cases, continued deficits in frontal white matter remain, perhaps due to abnormal lipid content of the white matter (Lesch et al. 1972, 1973). Although females are more susceptible to damage, females may have better recovery with abstinence than males (Lishman et al. 1987; Lishman 1990).

Further analysis of the white matter from alcoholics, moderate drinkers, and controls shows that cholesterol and phospholipid concentrations in the white matter are decreased in alcoholics compared to controls, in contrast to increased lipid content of white matter in moderate drinkers compared to alcoholics (Harper and Corbett 1990). Changes in the lipid content of white matter have been compared to the changes observed in degenerative diseases involving focal white matter, such as MS (Gallucci et al. 1989).

Although demyelination is not usually observed in alcoholics, except in cases of pontine myelinolysis, minor changes in myelin lamellae occur without death of neurons and glial cells. These changes, sometimes described as "myelin pallor," could account for reversible white matter shrinkage in alcoholics. However, not all minor white matter changes are reversible.

Even temporary changes may influence function, and some changes occur in moderate drinkers, with women having increased risk (Jacobson 1986). Mechanisms for producing myelin pallor followed by recovery are poorly understood. A similar condition in which brain weight is decreased without loss of neurons has been reported in cachectic individuals, who are suffering from nutritional wasting due to severe illness or emotional disturbance (Torvik 1987).

Unfortunately, there are few epidemiologic studies to describe the incidence of alcoholic brain damage (see Dufour, chapter 1), compared with those available on liver disease (Carlen and Wilkinson 1987*b*). Although white matter damage in some chronic alcoholics has been attributed to decreased blood flow resulting from hepatic dysfunction, white matter changes apparently occur frequently in individuals without overt alcoholic liver disease (see Harper and Kril, chapter 3).

Interest in the influence of ethanol on white matter in the brain has been renewed largely due to advances in technology that allow study of the brain in living individuals. Through advancements in brain scanning techniques, relatively noninvasive analyses of white matter in the brain can be made, allowing evaluation of the early effects of ethanol on the brain, even in social drinkers. Use of these techniques has provided valuable insight into individual sensitivities to ethanol and to differences in male and female responses to ethanol. These changes range from differences in brains of asymptomatic individuals to those observed in individuals with extensive motor and cognitive problems. An animal model in which ethanol was administered chronically to dogs has recently replicated human CT analyses showing disproportionate vulnerability of white matter, ventricular enlargement, glial cell loss, and variations in areas of neocortical susceptibility following chronic ethanol intake (Hansen et al. 1991).

Central Pontine Myelinolysis

Although white matter changes are frequently found in cases of severe alcoholism, true demyelination has most frequently been observed as central pontine myelinolysis (CPM) (Goebel and Herman-Ben Zur 1972; Vogel 1977; Harper and Kril, chapter 3). CPM has been associated with rapid correction of hyponatremia in chronic alcoholics (Tomlinson et al. 1976), in cases where ethanol was not involved (Tomlinson et al. 1976), and following heavy beer drinking of about 4 liters/day with decreased food intake (Papadakis et al. 1990). In the cases of hyponatremia, serum sodium levels as low as 96 to 100 mmol/L have been reported. Rapid correction of hyponatremia resulted in neurologic signs including quadriparesis, dysphasia, mutism, and loss of consciousness. Examination of the brain after death revealed myelinolysis in the central pons and extensive, similar lesions in both cerebral hemispheres. Myelinolysis was attributed to enhanced metabolic susceptibility of oligodendroglial cells in certain brain areas.

Risk for mortality from hyponatremia is increased 6- to 20-fold for patients with alcoholism (Papadakis et al. 1990), especially in patients with cirrhosis of the liver, where risk for hyponatremia may be as high as 48 percent for patients with severe encephalopathy. Abnormally high levels of arginine vasopressin, which may be present during ethanol withdrawal, could also contribute to brain edema and subsequent white matter damage observed as CPM and extra-pontine myelinolysis (EPM).

EPM lesions associated with CPM are often distributed symmetrically in the thalami, subthalamic nuclei, and the lateral geniculate bodies (Oda et al. 1984). In the deep layers of cerebral cortex, putamen, thalamus, and lateral geniculate, myelin cylinders form interlacing networks embedding large neurons and oligodendroglial cells. Reasons for the apparent enhanced susceptibility of oligodendroglia in these areas near neurons and other glial cells remain unclear.

More recent studies suggest that CPM occurs more frequently than previously thought. Moreover, death does not always occur in cases of CPM, and a mild form of CPM may be frequently undetected. Brain scans of five surviving patients with hyponatremia (four of the five patients had a history of alcoholism) have shown CPM (Pfister et al. 1985). Symptoms of these five patients ranged from severe tetraplegia and cranial nerve palsies to latent signs of pyramidal tract lesions and discrete ocular motor abnormalities. Pontine and extrapontine demyelination were confirmed neuroradiologically in two of the four alcoholic patients. An extrapontine lesion was observed in a third alcoholic patient, indicating that the incidence of demyelination associated with alcoholism is more frequent than previously thought, and that the clinical outcome is better than previously expected. Frequency of mild CPM is unknown, although the incidence in autopsied alcoholics has been estimated at 7 percent, cerebellar atrophy at 12 percent, and Wernicke's encephalopathy at 18 percent, (Riethdorf et al. 1991; Dufour, chapter 1).

Apparently, mechanisms for producing CPM are not uniform for all cases. For example, some individuals exhibit combined features of CPM and Marchiafava-Bignami disease, suggesting a common pathogenic mechanism. Marchiafava-Bignami disease has been described as degeneration of the corpus callosum in individuals consuming large amounts of red wine and may contain an allergic component (Ghatak et al. 1978).

Central spinal myelinolysis, including a midline lesion of the funiculus gracilis, has also been reported in individuals with a history of alcoholism, nutritional disturbances, and repeated episodes of electrolyte imbalance (Zwick et al. 1985). With new scanning technology, analysis of the lesions similar to CPM in the spinal cords of asymptomatic alcoholic individuals will be made possible.

CENTRAL NERVOUS SYSTEM CHANGES IN ANIMALS

Successful identification of animal models to mimic myelin changes associated with chronic ethanol intake by humans has been limited due to a variety of factors

associated with developing animal models for alcoholism. Long-term ethanol studies are expensive and difficult, because most animals will not drink ethanol voluntarily. This problem necessitates forced administration by a variety of techniques including ethanol in liquid diet, ethanol in drinking water, injection, inhalation, and gavage (Walker et al., chapter 11). In models where the animals are not drinking voluntarily, caloric or water intake may be reduced to avoid ethanol intake. Thus, undernutrition, dehydration, or stress associated with forced administrative techniques such as gavage or injection may confound results attributed solely to ethanol intake. Additionally, the species of animal chosen for study strongly affects reactions to ethanol.

Despite these difficulties, several chronic models have produced results confirming white matter damage associated with chronic ethanol use (Alling and Bostrom 1980; Alling 1983). Sun et al. (1980) reported reductions in myelin isolated from the brains of animals given ethanol chronically (15 percent ad libitum in liquid diet for 1 year) compared to controls. The myelin phospholipid/protein ratio was 20 percent lower, and the cholesterol/protein ratio was also lower (Sun et al. 1978) in animals given ethanol chronically for 1 year compared to controls. Furthermore, a 10-percent reduction in the phospholipid/protein ratio was observed in a group given ethanol by intubation for as little as 3 weeks. Changes in the phosphoglyceride acyl group composition, reflecting degenerative changes of myelin, were also observed.

When nutritional intake was considered, chronically administered ethanol, not impaired nutrition, decreased myelination in mice as measured by the myelin marker enzyme, 2',3'-nucleotide-3'-phosphohydrolase (Sedmak et al. 1978). Chronic ethanol administration to mice also resulted in significant inhibition of cerebral protein breakdown in whole brain and in subcellular fractions including myelin (Toth and Lajtha 1984). Decreased myelin cholesterol/protein ratio in the medulla oblongata of mice exposed to ethanol was attributed to destabilization of myelin membranes (Sun et al. 1978). However, others reported that intact myelin was less sensitive than synaptic membranes and extracted myelin lipids to the membrane-fluidizing effects of ethanol (Harris and Schroeder 1981; Hitzemann et al. 1989). These results suggest that some initial damage to the myelin may be required before progressive degenerative processes are established.

Decreased levels of lipid galactose of myelin in the cerebellum were reported in rats following chronic ethanol exposure for 6 months (Vrbaski and Ristic 1985). These results contrast with unaltered cholesterol content of myelin and synaptic membranes in swine given ethanol in beer for 3 years (Harris et al. 1983), suggesting the importance of species in determining vulnerability.

GLIAL CELLS

Although glial cells make up about one-half of the volume of the brain, until recently, glial cells gained little attention in the study of the response of the brain

to ethanol. Current research suggests that astroglia participate in neurotransmission. Astroglia have ion channels, receptors for neurotransmitters and hormones, and extensive processes that communicate with neurons and other glial cells (Kimelberg and Norenberg 1989; Teichberg 1991). Also, astrocytes maintain a certain amount of plasticity in their ability to respond to various injurious insults and can divide and proliferate throughout life. Thus, the role of these cells in the response of the brain to ethanol abuse is very important.

Ethanol and Glial Cell Changes in Specific Brain Areas

In studies of animals (Klemm and Engen 1978; Rosengren et al. 1985) and humans (Miyakawa et al. 1977), reports of gliosis following chronic ethanol use are common. Astrogliosis in frontal cerebral cortex and in cerebellar vermis of ethanol abusers has been reported. These findings correspond to reports of increased DNA without changes of the astroglial marker, S-100 protein; this indicates proliferative changes in glial cells in the anterior vermis of the cerebellum of gerbils exposed chronically to ethanol (Rosengren et al. 1985). In the posterior vermis of these animals, increases in S-100 protein and DNA suggest proliferation of astroglial cells. In the frontal cortex, S-100 protein levels were increased, but DNA decreased, indicating loss of cells and increased astroglial volume. These changes support human pathology reports of myelin pallor and gliosis associated with chronic alcoholism (Iwabuchi et al. 1990).

Ethanol increases manganese (Mn^{2+}) accumulation in glia, but not in neurons. However, some protection against ethanol damage in glial cells, but not neurons in culture, is afforded by Mn^{2+} treatment (Ledig et al. 1991). Such contrasting effects suggest different mechanisms for Mn^{2+} influence in glia and neurons (Tholey et al. 1990).

Glial cells provide enzymes for ethanol metabolism in the brain. Astroglia recently have been called the "liver" of the brain due to their enzymatic properties, ability to store glycogen, and release of lactate for energy (Hamprecht 1992). Neuronal cell bodies and astroglial and oligodendroglial cell bodies contain P450 IIE1, an enzyme that is active in ethanol oxidation. This enzyme is induced about tenfold in rat liver following ethanol treatment and participates in metabolic activation of precarcinogens, such as N-dimethyl-nitrosamines, carbon tetrachloride, and benzene (Hansson et al. 1990). In the neocortex, olfactory bulb, piriform cortex, and thalamic nuclei, P450 IIE1 is found in both neuronal and glial cell populations. In the cerebellum, P450 is found in all cell layers, but is located exclusively in the glial cells and their processes. Thus, glial cells may have an active role in metabolizing ethanol in the brain.

Ethanol and Glial Cells in Culture

Much current research is concentrated on the effects of ethanol on glial cells in culture. Ethanol retards differentiation (Waziri et al. 1981), and chronic ethanol treatment inhibits superoxide dismutase

activity, increasing vulnerability of the brain to free radical damage (Ledig et al. 1980; Mandel et al. 1980) (see Hunt, chapter 15, for a general discussion of free radicals and brain damage).

Ethanol exposure may interfere with glutamate metabolism by glial cells. Hypothetically, this interference could occur because ethanol decreases DNA and glutamine synthetase activity of astrocytes in culture (Davies and Vernadakis 1984). Depression of glial activity may be followed by a compensatory increase in glial-dendrite contacts and glial proliferation. Glial proliferation or damage would have profound effects on glial function in removal and metabolism of glutamate, GABA, and other neurotransmitters and neuromodulators from synaptic areas.

HYPOTHESIS FOR MECHANISM OF ALCOHOLIC DAMAGE TO WHITE MATTER

Alcoholism, like MS, has genetic and environmental components that have not been clearly explained. Although the triggering agent for MS has not yet been identified after many years of intensive research, presently there are some clues regarding the mechanisms and progression of the disease process which may provide insight into other diseases which are characterized by white matter damage, including alcohol-induced brain damage.

One characteristic of MS that leads to demyelination is the extreme sensitivity of oligodendrocytes to metabolic injury, such as hypoxia and altered electrolyte balance (Compston et al. 1991). Autoimmune damage to oligodendrocytes following injury may occur following T-cell sensitization and mediation of inflammation in response to cellular injury and/or infiltration of macrophages and other immune cells and factors through damaged areas of the blood-brain barrier. In response to injury and during oligodendrocyte repair, highly antigenic membrane vesicles are released. Antibody-independent complement activation is stimulated, thus attracting T cells, macrophages, and other immune cells, which may enter the central nervous system (CNS) through a compromised blood-brain barrier. These sensitized immune cells attack the surface of the oligodendrocyte and the myelin membrane (Sadler et al. 1991).

In MS, researchers have suggested that demyelination might not occur following single or multiple incidences of oligodendrocyte damage. However, in certain sensitive individuals or following repeated insults, autoantibodies and/or cells sensitized against oligodendrocytes and myelin membranes may lead to demyelination and a progressive autoimmune condition (Harris et al. 1991).

Alcoholic brain damage also involves damage to the white matter in brain, although the progress of the damage and resulting neurologic deficits may be milder than MS in the early stages. In the past, true demyelination was seen rarely in alcoholics, except in relation to pontine myelinolysis related to hyponatremia, rapid correction of hyponatremia, or other metabolic imbalances. However, new technology is showing that the influence of ethanol use on white matter has

been underestimated. The brain shrinkage and myelin pallor of asymptomatic drinkers must be addressed at the level of the oligodendrocyte and myelin membranes. Myelin damage and reactive astrogliosis associated with ethanol abuse are now apparent even in some asymptomatic individuals (Gallucci et al. 1989).

In some abstinent alcoholics, regression of the shrinkage, pallor, and plaque formation occur. In others, regression is slower and incomplete, and in some individuals, the active process of white matter damage progresses even in abstinence. Continued damage without the original causative agent suggests an autoimmune condition associated with alcoholic brain damage, and indeed, alcoholics appear to have a high incidence of autoimmune-related pathology (Schubert 1990). Damage to the blood-brain barrier associated with chronic ethanol abuse (Schafer 1985) would allow white cells into the nervous system, sensitizing the immune system to myelin and other white matter proteins resulting in consequent autoimmune activation.

In the event of oligodendrocyte or myelin damage by ethanol, microglia and perhaps astrocytes would become reactive and participate in removal of damaged cells and membranes via phagocytosis. These cells in turn act as antigen-presenting cells in association with major histocompatibility class I and class II antigens. T lymphocytes circulating through the area could become sensitized to the newly presented self-antigens resulting in activation of cytotoxic T cells. Cytotoxicity by T cells sensitized to self-antigens would set up an autoimmune response, resulting in tissue damage. If the blood-brain barrier is compromised, additional immune cells and factors not present normally in the CNS could enter the CNS in response to lymphokines released by T cells and microglia, resulting in a chronic, progressive, autoimmune reaction.

Ethanol enhances class I and II expression in a number of tissues, and the resulting increased presentation of antigens has been linked to some autoimmune pathologies associated with alcoholism (Singer et al. 1989). Tissues in which low levels of self-antigens are normally present include liver, kidney, and brain. Enhanced presentation of self-antigens in these areas through chronic ethanol exposure in sensitive individuals might result in an autoimmune reaction.

Increasing evidence illustrates an autoimmune component in certain individuals with alcoholic liver disease (Laskin et al. 1990). As with many autoimmune disorders, females have greater risk for these reactions and the resulting liver disease. Recent information shows increased expression of class I and II antigens in islet cells following ethanol exposure (Ruhland et al. 1991). These data support a hypothetical role for ethanol in the initiation and progress of the autoimmune reaction related to type I diabetes mellitus and suggest another important area of study.

Some interesting questions include whether the white matter is damaged first by ethanol, resulting in release of normally sequestered antigens and consequent immune sensitization, or whether the cytotoxic cells first become reactive to

enhanced presentation of autoantigens and respond with cytotoxicity (figure 1). Also, the relationship between autoimmune responses found in the liver and the CNS are unclear. Information showing improvement in cognitive function of alcoholic patients receiving liver transplants (Tarter et al., chapter 21) supports the importance of studying hepatocerebral connections as they may relate to brain damage in alcoholism.

SUMMARY AND CONCLUSIONS

Chronic ethanol intake is associated with decreased myelin in the brain evidenced by myelin pallor, plaque formation,

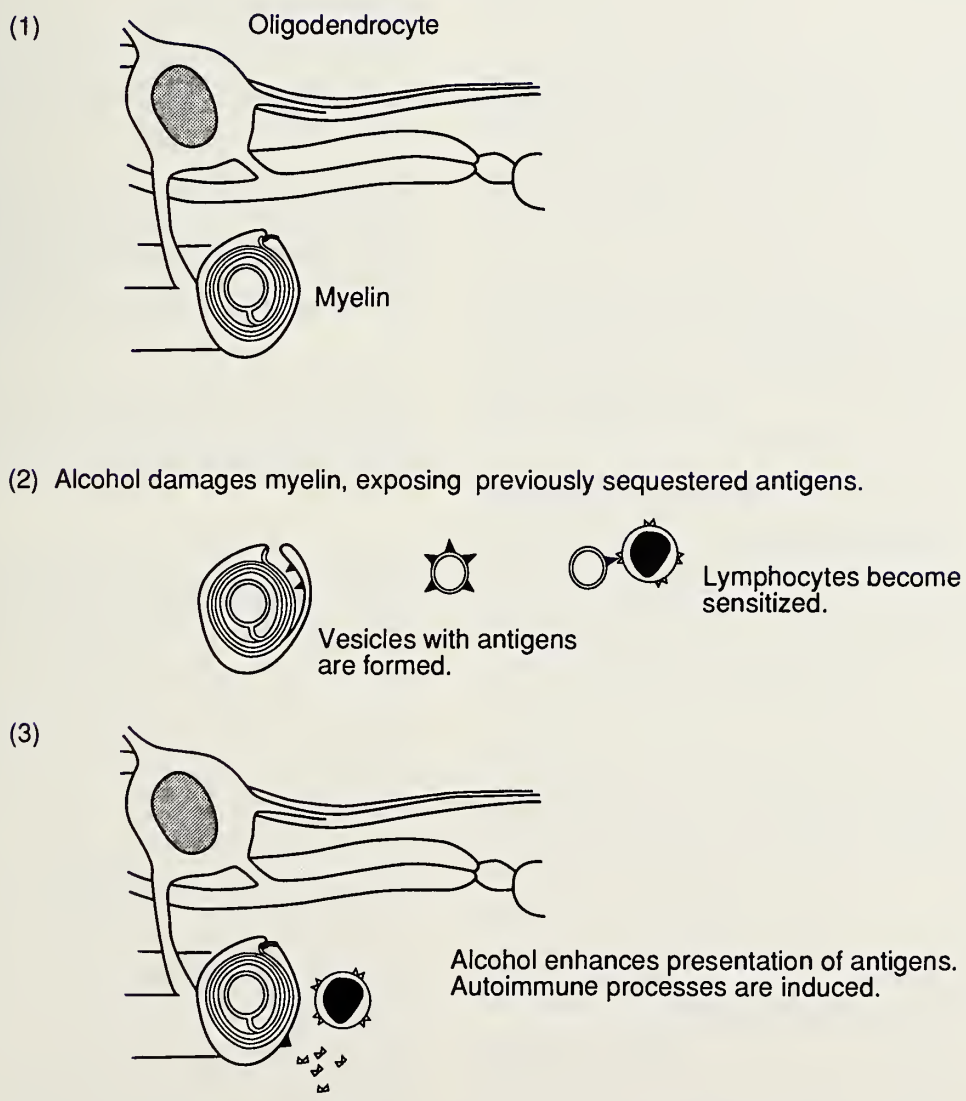

FIGURE 1

Autoimmune hypothesis. Scheme of a potential role for alcohol in the induction of autoimmune processes that may be involved in white matter damage associated with alcohol abuse. (1) Normal relationship of oligodendrocyte and myelinated axon in the CNS. (2) Alcohol damages myelin, resulting in the formation of vesicles bearing self-antigens and exposing previously sequestered self-antigens. T lymphocytes become sensitized to the self-antigens. (3) Alcohol enhances presentation of self-antigens associated with the major histocompatibility complex, increasing the chance for interactions with sensitized lymphocytes. Autoimmune processes are induced, and white matter is damaged.

ventricular dilatation, and overall shrinkage of the brain. Protein and water content of myelin increases in the alcoholic brain, corresponding to the loss of myelin lipids. Decreased cholesterol and phospholipid content are present as well as decreased cholesterol and phospholipid to protein ratios. These pathologies of white matter in chronic drinkers are more prevalent and occur earlier in the drinking history of females. The progression of white matter damage is more rapid in older drinkers as well. Moderate drinkers also exhibit abnormalities of white matter, shown as increased lipid content. The increased lipids are abnormal, however, and may be described as a compensatory response to the effects of ethanol. Damage to white matter and myelin may progress on a continuum throughout an individual's drinking history. Early in the drinking history, the individual may compensate for the effects of ethanol and, therefore, be asymptomatic. As the disease progresses, along with multiple insults, damaged myelin may sensitize the immune system, resulting in an autoimmune reaction. Thus, significant initial damage to myelin may be required for the degenerative process to proceed.

White matter, glial cells, and myelin are clearly vulnerable to the effects of ethanol. Apparently, white matter damage is more prevalent than previously thought. Damage to white matter caused by ethanol is dependent on many factors including the age of the individual at exposure, sex of the individual, chronicity of exposure, and nutritional status. Defects during bouts of drinking may regress, although occasionally some deficits may remain even during long-term abstinence. Continued expansion of our knowledge of the role of glial cells in neuronal function and development of techniques to regenerate neurons and astrocytes in adult brain (Reynolds and Weiss 1992) may lead to treatment of brain damage due to ethanol abuse in the future.

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20

A ROLE FOR NEUROTROPHINS IN ETHANOL-INDUCED BRAIN DAMAGE

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INTRODUCTION

Brain damage and functional abnormalities have been demonstrated in humans after chronic ethanol abuse. Similar alterations in behavioral, morphological, biochemical, and physiological measures of neuronal structure and function after chronic ethanol can also be found in controlled studies in laboratory animals (Walker et al., chapter 11; Hunter, chapter 13). However, we know little of the mechanisms underlying this ethanol neurotoxicity or which of the varied actions of chronic ethanol exposure are responsible for the cognitive deficits.

Rapid progress has been made during the past several years in our understanding of the well-known role of neurotrophic factors and their receptors in neuronal differentiation and survival. Understanding that neurotrophic factors can also play a critical role in cell differentiation and survival in the developing and mature central nervous system expands the potential functions of these molecules. Evidence already suggests that changes in neurotrophic factor production and responsiveness may occur in disease states, such as Alzheimer's and Parkinson's disease, in ischemia, and in epilepsy. Therefore, one important mechanism by which chronic ethanol exposure could produce neuronal toxicity is through a reduction in normal neurotrophic influences necessary for neuronal growth and survival. Chronic ethanol could exert this neurotoxic influence by decreasing the synthesis, availability, regulation, delivery, or biological activity of neurotrophic substances or by altering the capacity for target neurons to respond to these factors in a normal fashion. In this chapter, we will focus upon recent evidence regarding the functional role of neurotrophins in the central nervous system and preliminary studies in our laboratory designed to establish a role for these factors as a potential mechanism underlying ethanol neurotoxicity.

NEUROTROPHINS

A number of molecules have been shown to possess neurotrophic activity (Walicke

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1989), but few are synthesized in target neurons; that is, few are target-derived trophic factors. Nerve growth factor (NGF) is a prototype of a target-derived trophic factor that acts specifically on sympathetic and neural crest-derived sensory neurons in the peripheral nervous system (Johnson et al. 1986; Levi-Montalcini 1987). NGF plays a critical role in the development, survival, and maintenance of these neurons and is synthesized by them. In addition, NGF acts on specific receptors also synthesized by these neurons and transported to their terminals. The NGF receptors mediate local actions on the terminals, are bound by NGF, and are internalized and transported retrogradely to the cell bodies. Once there, they stimulate protein synthesis required for survival and expression of phenotypic characteristics of these neurons (Levi-Montalcini 1987; Johnson et al. 1987; Thoenen and Barde 1980). The NGF family of nerve growth factors has now become known as neurotrophins and currently has four additional members, including brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), neurotrophin-4 (NT-4), and neurotrophin-5 (NT-5) (Berkemeier et al. 1991; Hallbook et al. 1991; Leibrock et al. 1989; Maisonpierre et al. 1990).

Substantial evidence now supports the view that neurotrophins are required for the survival, function, and maintenance of neurons in the central nervous system, paralleling well-established actions in the peripheral nervous system (Springer 1988; Whittemore and Seiger 1987). Of potential importance for the neurodegenerative disease and cognitive dysfunction associated with chronic ethanol abuse and aging is that magnocellular cholinergic neurons of the basal forebrain, which send a cholinergic projection to the cortex and hippocampus, require neurotrophins, including NGF and BDNF, for survival and maintenance of normal function in culture (Alderson et al. 1990; Barde 1989).

A functional role for these neurotrophic factors has been considerably strengthened by studies in vivo. Basal forebrain neurons, which would ordinarily die following axonal transection, are rescued by neurotrophic factor replacement (Barde 1989).

Basal forebrain neurons may also be dependent upon neurotrophic factors that are not members of the neurotrophin family, particularly basic fibroblast growth factor (bFGF). bFGF is present in hippocampal neurons and has been shown to promote survival and growth of septal neurons in culture and in transection models in vivo (Pettmann et al. 1986; Grothe et al. 1989; Anderson et al. 1988). The significance of this apparent convergence of several neurotrophic factors on the survival and maintenance of a specific neuronal pathway is not yet known but implies the existence of relatively complex regulatory processes.

Considerable divergence may also occur among the multitude of neurotrophic factors already discovered, conferring substantial specificity to the action of individual molecules. In the periphery, recent studies have indicated that embryonic chick sympathetic ganglion cells are responsive to NGF but not to BDNF or

bFGF, whereas embryonic chick nodose ganglion neurons are not responsive to NGF or bFGF but are responsive to BDNF (Barde 1989). Such specificity is also likely to occur in the central nervous system. For example, NGF, BDNF, and bFGF all promote survival of rat septal neurons in culture (Grothe et al. 1989; Thoenen 1991). However, survival and neurite extension of rat hippocampal neurons in vitro is enhanced by bFGF but not by NGF (Mattson et al. 1989).

There has been significant progress in understanding the signal transduction process associated with neurotrophin receptor binding. NGF binding to neuronal receptors includes both low- and high-affinity components, and it appears that each may be required to support the biological actions of the neurotrophins. The low-affinity binding site is not sufficient to support the biological actions of NGF nor does it confer specificity among the neurotrophin family, since NGF, BDNF, NT-3, and NT-4 all bind this receptor with similar affinity (see Thoenen 1991; Lo 1992). Instead, the specificity of neurotrophin binding appears to be conferred by the high-affinity binding site.

Recent evidence indicates that the *trk* proto-oncogene encodes a high-affinity receptor for NGF and this receptor is a membrane tyrosine kinase (Kaplan et al. 1991). This high-affinity receptor tyrosine kinase appears to confer the specificity necessary to distinguish actions of members of the neurotrophin family. A *trkB* oncogene has now been found that encodes a receptor that binds BDNF, NT-3, and NT-4 with high affinity (Squinto et al. 1991), but not NGF. However, a *trkC* receptor tyrosine kinase has been reported to bind NT-3 with high specificity (Lamballe et al. 1991). A current view is that high-affinity, specific neurotrophin receptor binding involves a molecular complex that includes both the low-affinity, nonspecific binding site in combination with specific, *trk* receptor tyrosine kinase (see Lo 1992).

A significant feature of the link between the high-affinity binding site and the *trk* receptor family is the finding that the tyrosine kinase receptor is important in signal transduction mediating the actions of neurotrophins. Tyrosine kinases are rapidly activated after binding, and both NGF and BDNF have been shown to induce autophosphorylation (Kaplan et al. 1991; Soppet et al. 1991). In addition, tyrosine phosphorylation can stimulate a cascade of intracellular signalling pathways, including phospholipase C and mitogen-activated protein (MAP2) kinase (Vetter et al. 1991; Gomez and Cohen 1991). This process could explain the rapid response to changes in neuronal activity that have now been reported for the mRNAs encoding NGF and BDNF.

Experimentally induced seizures have been shown to substantially, but transiently, increase NGF and BDNF mRNA in hippocampus and neocortex (Ernfors et al. 1991; Isackson et al. 1991). In a recent comparative study, experimentally induced seizures were shown to dramatically increase BDNF mRNA in hippocampus and, to a lesser extent, NGF mRNA, whereas NT-3 mRNA was dramatically

reduced over the same period (Rocamora et al. 1992). Studies of neurons in culture have confirmed that NGF and BDNF mRNA can be rapidly altered (in 3 hours) by treatments that produce depolarization, particularly kainic acid or increased extracellular potassium (Zafra et al. 1990). These results provide preliminary indication that the neurotrophin family of growth factors may serve rapid and intermediary functions beyond their well-characterized actions on cell survival and differentiation. Such intermediary functions, including rapid responses to changes in impulse activity, could be influenced adversely by acute ethanol exposure. Continuous, chronic ethanol exposure could lead to enduring changes in neurotrophic factor function, ultimately leading to permanent changes in neuronal survival and cellular differentiation.

SEPTO-HIPPOCAMPAL CHOLINERGIC NEURONS: NEUROTROPHIC FACTORS

Neurotrophins appear to be required for survival, function, and maintenance of basal forebrain cholinergic neurons. The target areas of these neurons, the hippocampus and cortex, contain the highest levels of NGF protein and mRNA in the brain (Goedert et al. 1986; Whittemore et al. 1986). In situ hybridization studies indicate that NGF is synthesized in hippocampal pyramidal cells and granule cells of the dentate gyrus (Whittemore et al. 1988). When [^{125}I]-labeled NGF is infused into the hippocampus, retrograde transport of NGF to magnocellular neurons in the medial septal/diagonal band, but not to other hippocampal afferents, is observed (Schwab et al. 1979; Johnson et al. 1987). This finding suggests that specific NGF receptors are localized at the terminals of these neurons and that part of the signal transduction process involves internalization of the NGF/receptor complex, with subsequent retrograde transport. Monoclonal antibodies to the NGF receptor have been used to identify immunoreactive neurons in the rat, primate, and human brain. These neurons are distributed in the basal forebrain, with a location and morphology similar to that described for the magnocellular cholinergic neurons (Springer et al. 1987; Kordower et al. 1989; Mufson et al. 1989). Double-labeling studies have found more than 90 percent of the immunoreactive cells in basal forebrain are positive for both NGF receptor and choline acetyltransferase (Batchelor et al. 1989; Kordower et al. 1989). Northern blot analysis revealed the highest levels of NGF receptor mRNA in the basal forebrain area that has been confirmed by in situ hybridization studies (Ayer-LeLievre et al. 1988; Buck et al. 1987).

A variety of lesion studies have confirmed the role of neurotrophins in these cholinergic projections. Damage to the septohippocampal fibers elevates the level of NGF in the hippocampus, which is not accompanied by an increase in NGF mRNA (Gasser et al. 1987; Korsching et al. 1986; Collins and Crutcher 1985). The accumulation of NGF likely results from a loss of transport. Application of exogenous NGF to rat cholinergic neurons stimulates the expression of choline

acetyltransferase and promotes survival and fiber growth of forebrain cholinergic neurons both in vitro and in vivo (Gahwiler et al. 1990; Hagg et al. 1988; Hefti 1986). Finally, intraventricular administration of NGF promotes the survival or rescue of the basal forebrain cholinergic septohippocampal neurons axotomized by fimbria-fornix transection (Gage et al. 1988). This evidence supports the conclusion that the septohippocampal cholinergic neurons synthesize NGF receptors, which are then transported to the NGF-synthesizing target neurons in the hippocampus. When target-derived NGF is bound to the NGF receptor, it is internalized and transported retrogradely back to the NGF-sensitive cholinergic neurons.

There is also evidence indicating that age-related morphological and functional changes in basal forebrain cholinergic neurons may be due to deficient availability of NGF and decreased expression of the NGF receptor. Levels of NGF protein in the hippocampus are reduced by 40 percent in the hippocampus of aged rats (Larkfors et al. 1987). The NGF-binding capacity of the hippocampus and basal forebrain of aged rats is substantially reduced (Angelucci et al. 1988). The number and staining intensity of NGF-receptor immunoreactive neurons in the rat basal forebrain is also reduced (Gomez-Pinilla et al. 1989; Koh and Loy 1988). A consequence of reduced NGF receptor in the aged septohippocampal system is likely a decrease in the transport of NGF from the hippocampus to the basal forebrain cholinergic neurons. This decrease combined with the reduced availability of NGF in the target neurons in the aged hippocampus would deprive the basal forebrain neurons of normal neurotrophic support. Finally, a neurotrophic role for NGF is supported by the observation that the deficiency in memory and the atrophy of basal forebrain cholinergic neurons observed in aged rats can be ameliorated by chronic intraventricular infusion of NGF for 4 weeks (Fischer et al. 1987).

ETHANOL AND SEPTOHIPPOCAMPAL NEURONS

Chronic alcoholism with dementia has been reported to occur coincidentally with degeneration of cholinergic neurons in the basal forebrain and with reductions in choline acetyltransferase activity in the neocortex and hippocampus (Arendt et al. 1983; Nordberg et al. 1983; also see Arendt, chapter 22). A recent series of studies using rats has provided a strong link between impaired spatial memory dysfunction after chronic ethanol exposure, atrophy of hippocampal and basal forebrain neurons, and deficits in cholinergic function in the hippocampus and basal forebrain (Arendt et al. 1988, 1989; Hodges et al. 1991).

Among the magnocellular cholinergic neurons of the basal forebrain, the medial septal projections to the hippocampus were reported to be the more sensitive as compared to the nucleus basalis, the origin of a cholinergic projection to the neocortex. Moreover, we have also found evidence of deficits in the responses of cholinergic muscarinic receptors in the hippocampus after chronic ethanol

administration (Rothberg and Hunter 1991). These results have been extensively reviewed in previous chapters (see Walker et al., chapter 11; Hunter, chapter 13).

Arendt and coworkers have hypothesized that chronic ethanol exposure exerts a direct neurotoxic action on cholinergic neurons in the basal forebrain. The resulting degeneration of the cholinergic projections to the hippocampus and neocortex may underlie the deficits in spatial memory and the corresponding measures of hippocampal cholinergic synaptic function. Presumably, subsequent neocortical and hippocampal neuronal loss would occur by transynaptic cell loss, secondary to degeneration of septohippocampal cholinergic neurons. However, the basal forebrain neurons of the medial septal/diagonal band are dependent upon neurons in the hippocampus for neurotrophic support. Therefore, an alternative hypothesis requires the initial site of neurotoxic action of chronic ethanol to reside in the hippocampus, producing a loss or reduction of neurotrophic activity and a secondary degeneration of cholinergic septohippocampal projection neurons.

ETHANOL NEUROTOXICITY AND NEUROTROPHIC FACTORS

More than one mechanism is probably responsible for ethanol-induced brain damage. As noted above, one potentially important mechanism is that ethanol may reduce normal neurotrophic influences necessary for neuronal survival and growth. In view of the established link between neurotrophic factors and the structure and function of septohippocampal neurons, our laboratory has chosen to focus on this system as a model to investigate the role of neurotrophic factors in ethanol-induced neuronal damage.

We hypothesized that chronic, long-term ethanol exposure may cause a decrease in the synthesis, availability, upregulation, delivery, and/or the biological activity of normally occurring neurotrophic substances or may alter the capacity of target neurons to respond normally to these factors. Consequently, the structural and/or functional integrity of septohippocampal neurons may be compromised due to chronic deprivation of normal neurotrophic influence. One way in which this could occur is if ethanol reduced the availability of neurotrophic substances or target responsiveness on an acute basis, thereby exerting a cumulative deleterious action after continuous, chronic ethanol exposure. The influence of acute ethanol exposure on the survival and neurite outgrowth of dissociated dorsal root ganglion (DRG) cells in culture has been reported (Dow and Riopelle 1985). Low concentrations of ethanol (10 to 250 mg/dL) added to the culture medium significantly reduced the neurotrophic action of exogenous NGF on neurite outgrowth and partially blocked the neurotrophic activity produced in cell-conditioned medium.

Such a reduction in the availability of neurotrophic factors could serve a permissive role in allowing ethanol to exert cellular neurotoxic actions. We have examined the capacity of neurotrophic factors to protect against ethanol neurotoxicity. Our studies were conducted

using a tissue culture paradigm, in which neuronal populations were challenged with varying concentrations of ethanol. We found that neurotrophins appear to partially ameliorate ethanol toxicity. Cultured septal neurons, for example, respond to direct ethanol application in a dose-dependent manner, with a progressive decline in survival and process outgrowth with increasing ethanol concentrations. Neurotoxicity is considerably mitigated, however, by the presence of NGF in the culture medium. This finding is intriguing, because only a small subpopulation of these cells is normally NGF-responsive (i.e., cholinergic neurons of the medial septal and diagonal band of Broca nuclei). Thus, it is possible that the neurotrophins elicit *novel* responsiveness in normally unresponsive neurons. Such an effect has recently been shown in studies of hippocampal neurons (Cheng and Mattson 1991).

Hippocampal neurons normally do not respond to NGF. However, NGF appears to serve a protective role in response to neuronal injury induced by hypoglycemia (Cheng and Mattson 1991) or ischemia (Shigeno et al. 1991). We have also investigated the effects of varying NGF concentrations on the toxicity of ethanol on DRG neurons in vitro. We observed that low concentrations of ethanol (e.g., 250 mg/dL) are toxic to these neurons, if NGF concentrations are quite low (approximately 100 pg/mL). This toxicity is primarily reflected by a marked reduction in neurite outgrowth, as has been observed by previous investigators (Dow and Riopelle 1985). With increasing concentrations of NGF, however, the neurons can tolerate very high ethanol concentrations.

That neurotrophins serve a cytoprotective action is consistent with several recent observations. Hyman et al. (1991), for example, found that BDNF, but not NGF or bFGF, protects rat ventral mesencephalic dopaminergic neurons against 1-methyl-4-phenylpyridinium (MPP^+) toxicity. Cultured rat striatal neurons were protected by bFGF from N-methyl-D-aspartate (NMDA) receptor-mediated glutamate and quinolinic acid excitotoxicity (Freese et al. 1992). Intracerebral implantation in vivo of genetically engineered NGF-secreting fibroblasts has also been shown to similarly protect against excitatory amino acid neurotoxicity (Schumacher et al. 1991). We are currently extending our in vitro investigation of ethanol neurotoxicity by developing methods that will enable us to identify specific phenotypes of cultured neurons, analyze in detail their responsiveness to ethanol, and explore the mechanisms underlying the neurotrophic factor-induced attenuation of neurotoxicity.

Prolonged exposure to ethanol could alter the capacity for neurotrophins to function properly by still more complex interactions. For example, alterations in target-derived neurotrophic substances by chronic ethanol exposure could alter the integrity of specific target populations and compromise neuronal cytoprotection in a highly selective pattern. Our initial approach to studying chronic ethanol actions on the neurotrophins has involved the use of dissociated embryonic chick

DRG cells in culture as a bioassay to determine if chronic ethanol exposure reduces the neurotrophic activity contained in rat hippocampus. These cells are normally dependent upon neurotrophins for cell survival and neurite outgrowth. When exposed to media free of NGF but containing hippocampal extracts, these cells exhibit normal survival and neurite outgrowth. We have found that 20 to 24 weeks of chronic ethanol exposure produced a 50-percent reduction in the neurite-promoting neurotrophic activity contained in hippocampal extracts as compared to extracts from controls (Walker et al. 1992*b*). Hippocampal extracts were prepared 48 hours after chronic ethanol treatment was terminated to ensure that ethanol was absent from the extracts at the time of bioassay.

More recently, we have compared the extent of neurite-promoting activity contained in hippocampal extracts prepared 4 to 5 months after chronic ethanol exposure and control diets were withdrawn. Hippocampal extracts from rats chronically exposed to ethanol contained significantly less (25 percent) neurite-promoting activity than hippocampal extracts prepared from pair-fed control rats despite long-term abstinence (Walker et al. 1992*a*). The neurotrophic activity contained in the hippocampal extracts was neutralized in the presence of antibodies against NGF but was unaffected by antibodies against bFGF, suggesting that the neurotrophic activity, measured by the DRG assay, is predominately NGF-like. The chronic ethanol-induced reduction in hippocampal neurotrophic activity was entirely reversible with the addition of NGF to the culture media. This reversibility by NGF demonstrates that the reduction in neurite outgrowth in the presence of hippocampal extract from ethanol-treated rats does not result from the production of inhibitory or toxic factors during chronic ethanol treatment.

CONCLUSIONS

These results indicate that chronic ethanol exposure reduces significantly and persistently the neurotrophic activity in the hippocampus. A major proportion of the neurotrophic activity appears to be NGF-like. Since neurons in the medial septal nucleus are dependent on neurotrophic influences from the hippocampus for survival and growth, the observed decrease in hippocampal content of neurotrophic activity may compromise the structural and functional integrity of these neurons. Whether the reduction in neurotrophic activity is linked exclusively to NGF awaits more conclusive direct measurements of the concentration of NGF and its corresponding mRNA. Such studies are ongoing in our laboratory and are particularly critical. Medial septal neurons may be dependent upon other members of the neurotrophin family as well as other neurotrophic factors, including bFGF.

One approach that has proven fruitful in dissecting differential actions of neurotrophic factors is a comparison of the neurotrophins in bioassays using several cell lines. For example, recent studies have indicated that embryonic chick sympathetic ganglion cells are responsive to NGF but not to BDNF or bFGF (Barde

1989; Maisonpierre et al. 1990). On the other hand, embryonic chick nodose ganglion neurons are not responsive to NGF or bFGF but are responsive to BDNF (Barde 1989). It should also be recalled that while NGF, bFGF, and BDNF all promote survival of rat septal neurons in vitro (Grothe et al. 1989; Thoenen 1991), survival and neurite extension of rat hippocampal neurons in vitro is enhanced by bFGF but not by NGF (Mattson et al. 1989). For these reasons, parallel studies are necessary using several additional cell lines to determine if hippocampal extracts promote survival and neurite outgrowth of cultured sympathetic ganglion, nodose ganglion, and septal and hippocampal neurons; if this neurotrophic activity is reduced by chronic ethanol treatment; and if the activity can be blocked by various neurotrophin antibodies. These studies should provide important additional controls for this bioassay approach and a powerful source of options for inclusion or exclusion of different neurotrophins in the action of ethanol. Since the *trk* high-affinity tyrosine kinase receptor differentiates among members of the neurotrophin family, immunohistochemical studies using the newly available antibodies to *trk* and *trkB* may also illuminate a differential role of neurotrophins in ethanol neurotoxicity.

Classically, the neurotrophins have been considered as molecules important in neuronal survival and differentiation. However, recent studies have emphasized potential novel actions of neurotrophic factors. Thus, NGF can serve a cytoprotective action in hippocampal neurons in response to hypoglycemia in vitro (Cheng and Mattson 1991) or ischemia in vivo (Shigeno et al. 1991). This action has been demonstrated despite the fact that NGF does not ordinarily support cell survival of hippocampal neurons in culture (Mattson et al. 1989). The evidence, coupled with rapidly occurring regulatory changes in the neurotrophins in response to neuronal impulse activity, supports a broader view regarding the function of the neurotrophins in the adult nervous system. Our finding that NGF can also protect against ethanol neurotoxicity in cell culture lends further support to this view. Thus, the ability of chronic ethanol exposure to produce persistent reductions in neurotrophic activity even after prolonged periods of abstinence (i.e., 5 months) could render neurons in many brain areas vulnerable to a variety of neurotoxic insults, including hypoglycemia, ischemia, and even renewed ethanol consumption.

The acute actions of ethanol on neuronal survival are complex and dose dependent. At quite low concentrations, ethanol may serve a protective function against excitotoxic insults, perhaps by its ability to reduce transmembrane calcium fluxes through NMDA or calcium channels (see Crews and Chandler, chapter 17). However, at higher concentrations in tissue culture models ethanol becomes neurotoxic. The concentration-dependence of this latter action of ethanol can be modulated or gated by the concentration of neurotrophins present in the media. The mechanism underlying this action of neurotrophins in protecting against ethanol neurotoxicity is not yet known. However,

the ability of the neurotrophins to protect against hypoglycemic/excitotoxic insults might result from a stabilization of neuronal calcium homeostatic mechanisms (Cheng and Mattson 1991).

Therefore, a point of important convergence may exist between the mechanisms regulating intracellular calcium homeostasis, acute ethanol actions on membrane channels important in transmembrane calcium delivery, and acute and chronic ethanol actions on neurotrophin availability and receptor function. It appears that we must add the neurotrophins and associated signal transduction processes (see also Shanley and Wilce, chapter 14) to the already complex interaction between acute ethanol exposure and calcium regulatory processes important in acute intoxication as well as chronic ethanol neurotoxicity. Future studies must address the role of each of these important molecules in the neurotoxic actions associated with prolonged ethanol exposure.

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21

LIVER-BRAIN INTERACTIONS IN ALCOHOLISM

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INTRODUCTION

Alcoholics commonly exhibit a variety of deficits on neuropsychologic tests that measure cognitive capacity. These impairments undoubtedly have a multifactorial etiology. Certain of the cognitive deficits may presage the onset of alcoholism and likely comprise a vulnerability factor for developing this disorder (Tarter et al. 1984*a*, 1989). Other aspects of their cognitive impairment emerge concomitant to chronic excessive alcohol consumption (Tarter and Edwards 1985).

The neuropsychologic disturbances concomitant to chronic alcoholism are the consequence of two interrelated processes: addiction and toxicity from chronic ethanol exposure together with associated systemic disturbances. Addiction following habitual alcohol consumption results in neuroadaptational changes within the central nervous system (CNS) involving neurochemical and neurophysiological processes. Little is known about these neurobiological mechanisms with respect to cognitive functioning. Thus, the extent to which impaired cognitive functioning commonly observed among alcoholics is the result of addiction-related alterations of brain functioning remains unknown.

With respect to ethanol toxicity and associated organ system injury, it is noteworthy that controlled research on animals has shown conclusively that alcohol is a neurotoxin (see other chapters in this section and the first section). However, the specific contribution of ethanol neurotoxicity to the broad array of neurologic disturbances manifested by alcoholics cannot be precisely determined. One reason for this is that other organ systems may be adversely affected by alcohol, and these systemic disruptions may also be associated with cognitive deficits. Hence, to delineate the pattern and severity of neurologic disorders concomitant to the exclusively human clinical condition of alcoholism, it is necessary to conduct broadly based, multivariate research in which several etiologic factors are concurrently considered.

This chapter examines liver-brain interactions in alcoholism. Acute liver

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failure and chronic, advanced liver disease may produce neuropsychiatric disturbances that generically are called hepatic encephalopathy. Cirrhosis is among the more frequent medical consequences of alcoholism and is a necessary condition for producing a portal systemic encephalopathy. This type of encephalopathy is caused by intrahepatic and extrahepatic shunting of venous blood, such that the liver cannot perform its filtering functions. There is considerable reason to believe that certain aspects of the neurologic pathology observed among alcoholics with liver disease, specifically those with cirrhosis, may be the manifestation of a coexistent hepatic encephalopathy.

The ensuing discussion consists of a brief overview of the functions of the liver and the acute and chronic variations of hepatic encephalopathy. Next, the contribution of hepatic encephalopathy to the overall neurologic pathology observed in chronic alcoholics is examined. Finally, the implications of this research for improving the medical management of alcoholics for possibly ameliorating the neurologic pathology are addressed.

FUNCTIONS OF THE LIVER

The liver, the largest internal organ, is responsible for more metabolic functions than any other organ. Communication with the brain occurs via the vascular system. Briefly, the main functions of the liver are described below and consist of the following activities: filtration and excretion, metabolism, immune defense, hematologic function, and bile production.

All substances absorbed by the intestines are transported to the liver through either the portal venous system or via the lymphatic and then to the systemic circulation. These substances include biologically important nutrients as well as toxins. A major function of the liver is to trap and degrade toxic endogenous and exogenous compounds that may be potentially hazardous. These substances require hepatic metabolism before biliary and urinary excretion. When the liver is cirrhotic, and venous blood is shunted around the liver, this filtering function is compromised. Therefore, neurotoxins, such as ammonia and a wide array of neurogenic amines, are retained in the circulation and may be transported into the brain. Once there, they contribute to the pathogenesis of hepatic encephalopathy.

The synthesis, metabolism, and storage of energy substrates are regulated by the liver. During episodes of fasting, the liver can regulate the release of macronutrients (i.e., fat, carbohydrate, and protein) into energy. The liver is a major depot for certain vitamins and is the primary storage site for carbohydrates. The liver also inactivates steroid hormones, such as estrogens, progesterone, and androgens.

Kupffer cells, located in the liver, remove bacteria and other macromolecular substances that are present in portal venous blood. The liver regulates their availability to the immune system. In addition, the liver manufactures many different coagulation factors that regulate clot formation and dissolution.

Moreover, in periods of stress the liver can function as a blood cell-producing organ. The liver also produces bile acids that are essential for the digestion and absorption of fat in the diet and for the excretion of hydrophobic materials.

The variety and complexity of the functions of the liver illustrate the importance of this organ for maintaining health status in general and neurologic integrity in particular. Recognizing that the brain is virtually dependent on the functional integrity of other organ systems on a moment-to-moment basis to maintain its optimal functioning and viability, a disruption of hepatic function evidently is paralleled by a disruption of neurologic functioning. Hepatic encephalopathy is the general term applied to the constellation of neuropsychiatric disorders that occur as a consequence of end stage liver disease. The integral involvement of the liver with respect to nutrition, energy production and use, detoxification, and immune functions underscores the complex processes that are potentially involved in the pathogenesis and expression of hepatic encephalopathy. (For a more comprehensive description of hepatic physiology the reader is referred to Zakim and Boyer 1990.)

HEPATIC ENCEPHALOPATHY

Acute Hepatic Encephalopathy

Sudden and severe hepatic failure produces an encephalopathy that has a primarily metabolic etiology. With effective treatment of the liver disease, this form of encephalopathy is reversible. Therefore, the severity of acute hepatic encephalopathy covaries with the severity of the liver disease. Table 1, adapted from Conn and Lieberthal (1978), summarizes the neuropsychiatric signs and symptoms of acute hepatic encephalopathy.

Hepatic encephalopathy is graded from zero to four according to observational findings and results obtained from an examination of the mental status of the patient (Zieve 1979). A rating of zero is assigned if no disturbances in mental status, emotion, behavior, or neurologic impairment are detectable. This grade does not necessarily imply that neuropsychiatric disturbances are not present; rather it means that they are beyond detection employing usual examination procedures. As discussed subsequently, a zero rating does not preclude the possibility of a latent or subclinical encephalopathy. Standard neuropsychologic tests commonly reveal neurocognitive and psychomotor impairment in these individuals (Rehnstrom et al. 1977; Tarter et al. 1984*b*).

Disturbances in attention span, concentration, and vigilance are among the first overt indications of hepatic encephalopathy (grade 1). Tasks requiring mental effort, such as arithmetic computations, are performed poorly. Both as the result of the direct effects of the neuropsychiatric disturbance and the person's cognizance of their inability to optimally exercise cognitive capacities, anxiety, apprehension, and worrisome preoccupation may appear and further compromise cognitive functioning. Accompanying these symptoms are mood shifts that

TABLE 1

Progression of neuropsychiatric disturbance associated with acute episode of portal systemic encephalopathy

	Severity grade of encephalopathy				
	0 (Normal)	**1 (Mild)**	**2 (Moderate)**	**3 (Severe)**	**4 (Coma)**
Consciousness	No detectable change	No clear impairment	Mild disorientation	Confusion/ stupor	
Activation	Normal	Inversion of sleep pattern and/or insomnia/hypersomnia	Inversion of sleep pattern and/or insomnia/hypersomnia	Somnolence	
Behavior	Normal	Personality change Fatigue	Lethargy Disinhibition Inappropriateness	Bizarreness Depersonalization Paranoia	
Affect	Normal	Irritability Euphoria or depression	Anxiety Anger	Rage	
Cognition	Normal	Attention deficit Concentration difficulties	Impaired time sense	Amnesia	
Neurological	Normal	Tremor Incoordination	Ataxia; asterixis Slurred speech Hyperactive reflexes	Dilation of pupils Hyperactive reflexes Rigidity Nystagmus	

occur suddenly and dramatically. These mood changes range from euphoria to depression. Irritability and fatigue are also observed frequently. Standard clinical neurologic examination typically reveals difficulties in motor coordination and fine motor control.

If not accurately diagnosed, or if ineffectively treated, the encephalopathy typically progresses in severity. Moderate or grade 2 severity features the emergence of disorientation with respect to time and place. Heightened anxiety, intense expression of emotions (especially anger), apathy, and inappropriate behavior are common features. Disinhibition of both neurologic and behavioral processes are also pronounced features of this stage, resulting in hyperactive reflexes and diminished behavioral and affective control. In addition, dysarthria is commonly present and manifests as slurred and poorly articulated speech.

Progression to grade 3 hepatic encephalopathy is potentially life threatening. Sleep disturbances, which are

sometimes present in grades 1 and 2, may progress to prolonged periods of somnolence. Consciousness is grossly impaired, and the pattern of disturbance is typically delirium or stupor. Consequently, behavior is bizarre and unpredictable. Delusions, outbursts of rage, and depersonalization are also common features. Neurologic functioning is globally disrupted and characterized by pervasive cognitive impairment, hyperactive reflexes, rigidity, nystagmus, ambulatory incapacity, and dilation of the pupils. Without effective medical intervention, the individual lapses into coma or grade 4 encephalopathy.

Chronic Hepatic Encephalopathy

Cirrhosis is frequently associated with a low-grade chronic hepatic encephalopathy (Jones and Gammal 1988). Alcohol consumption, being the most common cause of chronic liver disease in Western societies, where alcohol beverages are readily available, is the principal etiologic determinant of this variant of hepatic encephalopathy. Portal systemic encephalopathy (PSE) is the term used to describe the neuropsychiatric disorder that occurs because of shunting of portal venous blood around a diseased liver. The causal relationship between cirrhosis and PSE is well established (Conn and Lieberthal 1978). As a result of shunting, putative neurotoxic substances remain in the circulation and initiate the pathophysiology of PSE. According to Zieve (1979), however, there are numerous factors that can contribute to the clinical expression of portal systemic encephalopathy. These factors include (1) decreased brain glucose and oxygen consumption; (2) increased ammonia levels in blood, brain, spinal fluid, and muscle; (3) increased glutamine levels in the brain, spinal fluid, and muscles; (4) increased short-chain fatty acid levels in the blood and possibly also the brain; (5) decreased neurotransmitter levels in the brain; (6) increased "false" neurotransmitter levels in the brain, blood, urine, and muscle; (7) increased organic mercaptan levels in the brain, blood, breath, and urine; (8) altered amino acid ratios; (9) decreased affinity of hemoglobin for oxygen; and (10) increased concentration of neurotransmitter metabolites in brain and spinal fluid.

Thus, neurotransmitter dysregulation, depression of brain energy, and the accumulation of neurotoxins, each acting independently in concert, appear to be important determinants of PSE. Although still controversial, it is thought that increased γ-aminobutyric acid (GABA) levels may play a role in the pathogenesis of hepatic encephalopathy (Jones and Skolnick 1990). Furthermore, recent studies have also found an important role for certain nutritional deficiencies (i.e., vitamins A and E) as factors contributing to psychomotor disturbances associated with PSE (Arria et al. 1989, 1990*a*).

Chronic, low-grade hepatic encephalopathy can persist as a stable neuropsychiatric condition or flare into a fully developed, acute encephalopathy during episodes of medical decompensation. As long as the critical etiologic factor of cirrhosis is present, an encephalopathic

process of variable degree will be present. The implications for alcoholism are noteworthy. Even after cessation of alcohol consumption, the presence of alcohol-related cirrhosis may sustain the encephalopathy, thereby compromising the neurologic functioning of remitted chronic alcoholics with cirrhosis.

It is important to emphasize that hepatic encephalopathy is a metabolic disorder that occurs because the liver is not functioning normally. Although each individual episode of hepatic encephalopathy is potentially reversible, it remains to be determined whether repeated episodes have a cumulative effect and ultimately cause permanent neurologic injury.

An acute encephalopathic episode can be precipitated by several factors in patients who have a chronic, low-grade encephalopathy. In patients with cirrhosis, Conn and Fessel (1971) found that the triggering events for an acute episode of hepatic encephalopathy in decreasing order of frequency were azotemia (presence of nitrogen-containing substances in the blood, such as urea or ammonia), administration of sedatives or analgesic drugs, gastrointestinal hemorrhage, hypokalemic alkalosis, an excess dietary intake of protein, infection, and constipation. Azotemia and gastrointestinal bleeding alone accounted for about 50 percent of episodes. Significantly, both disorders commonly accompany alcoholism.

HEPATIC ENCEPHALOPATHY COEXISTENT WITH ALCOHOLISM

Systematic research into the contribution of hepatic encephalopathy to the overall pattern of neurologic disturbances displayed by alcoholics has only become possible recently. Prior investigations have been hampered by the relatively low prevalence and, hence, unavailability for study of individuals with nonalcoholic cirrhosis. This problem makes it difficult for investigators to accrue the necessary control subjects with nonalcoholic liver disease for comparison to alcoholics. In addition, without an effective treatment to restore the person to a state of normal liver function, it has not been possible to differentiate the specific neurologic effects attributable to liver disease per se from those disturbances caused by disruption of other organ systems.

The large liver transplantation program at the University of Pittsburgh obviates these problems. This clinical service attracts many individuals with cirrhosis having a variety of etiologies (i.e., viral, autoimmune, or alcohol). Furthermore, the transplantation program routinely evaluates individuals who ultimately do not qualify for liver transplantation, because the disease either is not advanced enough or has advanced to a stage where the procedure cannot be performed with any reasonable hope of success. Thus, patients with alcoholic and nonalcoholic liver diseases, having a very broad spectrum of disease severity, are available for study.

The ability to compare alcoholics and nonalcoholics affords the opportunity to determine the influences of liver disease type and severity on the neurologic disturbances present. Also, because the 1-year postoperative survival rate approaches 90 percent (Van Thiel et al. 1991), it is possi-

ble to quantify the changes in neurologic status that occur following a successful liver transplantation. Therefore, by comparing matched alcoholic and nonalcoholic cirrhotic subjects before and following hepatic transplantation, and by contrasting the status of alcoholic cirrhotics before and after surgery, it is possible to draw conclusions about the specific contributions of liver disease and alcoholism on the manifold neurologic disturbances.

Neuropsychologic Findings

Gilberstadt et al. (1980) compared the neuropsychologic test performance of alcoholics having biopsy-confirmed cirrhosis to alcoholics without cirrhosis. None of the subjects had evidence of overt encephalopathy. Each subject was administered portions of the Wechsler Adult Intelligence Scale (WAIS) and the Trail Making Test (a visuomoter speed test). Compared to noncirrhotic alcoholics, subjects with cirrhosis were impaired on tests measuring visuospatial scanning, spatial organization, writing speed, and reaction time. Whereas verbal IQ was comparable between the two groups, performance IQ was almost 10 points lower in the cirrhotic subjects. Fifty percent of the cirrhotic subjects obtained scores on at least one test that were more impaired than any of the noncirrhotic, alcoholic controls. In another study, Smith and Smith (1977) observed no differences on the WAIS verbal IQ score between cirrhotic and noncirrhotic alcoholics. However, confirming the results of Gilberstadt et al. (1980), they observed that the performance IQ was inferior in cirrhotic alcoholics, compared to noncirrhotic alcoholic controls.

The above studies indicate that the presence of cirrhosis in alcoholics is associated with a greater range and severity of neurocognitive deficits than is seen in noncirrhotic alcoholics. Although implicating an adverse effect of cirrhosis on neurocognitive capacities, it should be noted that these results were potentially confounded by other important but uncontrolled factors. For example, the cirrhotic alcoholics may have been abusing alcohol longer, and their liver disease may not be the only etiologically relevant factor relating to their test performance. Also, disruptions in other organ systems and nutritional deficiencies may have contributed to the observed greater neuropsychologic impairments seen in the alcoholic cirrhotics. For these reasons, the inclusion of a comparison group of nonalcoholic cirrhotics is essential for understanding the impact of cirrhosis, which may be separable from alcoholism per se.

Alcoholics with Cirrhosis Compared to Nonalcoholics with Cirrhosis

Because cirrhosis is a controlled variable, contrasting alcoholics and nonalcoholics who have cirrhosis affords the opportunity to determine the impact of alcoholism on neuropsychologic capacity. There is a strong degree of similarity in the test performance of alcoholics and nonalcoholics with biopsy-confirmed cirrhosis (Rehnstrom et al. 1977; Rikkers et al. 1978; Tarter et al. 1988). Specifically, impairments in the same domains of cognitive functioning are found among cirrhotic

alcoholics and nonalcoholic cirrhotics. Also detected were deficits on tests of nonverbal intelligence, abstracting capacity, learning and memory, visuospatial processes, praxic abilities, and on tests of psychomotor efficiency.

In a recent study, Tarter et al. (1988) found that alcoholics and nonalcoholics with biopsy-confirmed cirrhosis performed similarly on a battery of approximately two dozen neuropsychological tests. The alcoholics performed more poorly, however, on several tests, suggesting an effect of alcoholism on certain cognitive functions beyond those attributed to cirrhosis. Nonetheless, because most of the tests did not discriminate the cirrhotic alcoholics from cirrhotic nonalcoholics, it was concluded that liver disease, particularly cirrhosis and its sequelae, was the major influence on neurocognitive disturbances found in chronic alcoholics. Rehnstrom et al. (1977) reported similar findings and observed that the performance of cirrhotic alcoholics resembled noncirrhotic alcoholics on tests of memory, attention, language, visuospatial and psychomotor capacity, and intelligence.

In a study designed specifically to evaluate the role of cirrhosis on memory capacity, Arria et al. (1991*b*) compared four groups of subjects (normals, noncirrhotic alcoholics, cirrhotic alcoholics, and cirrhotic nonalcoholics) on a battery of memory tests. Not surprisingly, the normal controls performed better than the other three groups. However, the noncirrhotic alcoholics and normals performed better than both alcoholics and nonalcoholics with cirrhosis. Moreover, on the Brown-Peterson task, a demanding and sensitive measure of short-term memory capacity, the alcoholic cirrhotics were most impaired, followed by the nonalcoholic cirrhotics and the noncirrhotic alcoholics, respectively. Thus, although a history of alcoholism is associated with neurocognitive disturbances, much (but not all) of the impairment seen in alcoholic cirrhotics may be explained by a coexistent, chronic, low-grade hepatic encephalopathy.

Neuroradiologic Findings

Neuroimaging studies also suggest an important effect of chronic liver disease on the gross anatomy of the CNS in alcoholics. (For a general discussion of neuroimaging, see Pfefferbaum and Rosenbloom, chapter 4.) Measurements of brain morphology using computerized tomography (Bernthal et al. 1987) and magnetic resonance imaging (Barthauer et al. 1992) show that the presence of cirrhosis in alcoholics is associated with augmented cerebral atrophy. One study showed a covariation between severity of liver disease and magnitude of cerebral atrophy (Acker et al. 1982). In another study, alcoholics and nonalcoholic cirrhotics were more similar to each other than they were different (Barthauer et al. 1992). Where blind ratings of cortical atrophy were obtained, greater pathology was noted in the frontal and cerebellar regions in alcoholic cirrhotics compared to nonalcoholic cirrhotics.

Using digitized photography, de La Monte (1988) measured several regions of the cerebrum, cerebral cortex, subcortical

nuclei, cerebral white matter, and ventricles in post mortem brain tissue of alcoholic and nonalcoholic cirrhotics. Reductions in cross-sectional areas of the cerebrum and disproportionate atrophy of white matter were found in the alcoholic group compared to the nonalcoholic group. There was no evidence of abnormalities in subcortical nuclei. The degree of ventricular enlargement was similar to the degree of white matter atrophy, suggesting that compensatory hydrocephalus accounted for the increase in ventricle size. The results of this study suggested that axonal degeneration, evidenced by disproportionate atrophy of white matter, may be more severe in alcoholics with liver disease compared to individuals with nonalcoholic liver disease.

Thus, the imaging studies complement the findings of the neuropsychologic studies. Specifically, cirrhosis appears to contribute to the severity of gross anatomic injury of the brain in the alcoholic, although it should be emphasized that it does not account for all of the pathology. More studies are needed in which cerebral atrophy is measured directly using noninvasive imaging technologies and correlated with performance deficits in alcoholics at various stages of liver disease.

Physiologic Findings

Greater electroencephalography (EEG) slowing is seen in alcoholics with cirrhosis compared to alcoholics without cirrhosis (Kardel and Stigsby 1975; Kardel et al. 1972). Also, a greater reduction in cerebral blood flow has been observed in alcoholics with cirrhosis compared to nonalcoholic cirrhotics (O'Carrol et al. 1991; Lockwood et al. 1991).

CORRELATION BETWEEN BIOCHEMICAL MEASURES OF LIVER AND NEUROPSYCHOLOGIC PERFORMANCE

Preliminary evidence has been reported implicating an association between the severity of biochemical measures of liver injury and function and neuropsychologic test performance (Tarter et al. 1986; Schomerus et al. 1981; Gilberstadt et al. 1980). It should be pointed out, however, that the results are not consistent across all studies. In some studies, for example, plasma ammonia levels (a neurotoxin causally implicated in PSE) were negatively correlated with neuropsychologic test performances. In other studies they were associated with other measures of hepatic injury. This inconsistency among investigations may be due to many factors, including differences in sampling strategies, time of measurement, and type of neuropsychologic measures used.

The discrepancy among the various studies highlights the complexity of the association between hepatic function, hepatic failure, and PSE. Large-scale, multivariate research with multiple variables of hepatic injury and function, singly and in combination, is needed to explain or account for neuropsychologic test score variance. In one such study, Moss et al. (1992) found that the different aspects of liver injury and function were related to certain neuropsychologic impairments. In this latter study,

impaired nitrogen detoxification was associated with slow perceptual speed, impaired visuopractic capacity, and psychomotor slowing. On the other hand, disturbed protein synthesis and intrahepatic blood flow were related to language-based deficits. To evaluate the relation of hepatic injury to neurologic function in alcoholics, some investigators have studied noncirrhotic alcoholics recruited from clinical treatment facilities. One study found a significant correlation between γ-glutamyl transpeptidase (GGTP, a standard measure of liver injury) and neuropsychologic test performance in noncirrhotic alcoholics (Irwin et al. 1989). Because GGTP is also a fairly good indicator of recent drinking, it was difficult to discern whether it was the liver injury or a recent history of alcohol consumption that was accounting for the change in neuropsychologic performance. More recent work by Richardson et al. (1991) expanded on these findings by studying detoxified alcoholics 21 days after their last drink. Alcoholics with biochemical evidence of liver injury (measured by elevations in GGTP and other liver enzymes) performed more poorly on tests of visuospatial and conceptual capacities compared to alcoholics with only mild elevations or normal GGTP levels.

Finally, Schaefer et al. (1991) attempted to determine the relative power of several variables for predicting cognitive performance in alcoholics. Drinking history, depression, liver function, nutrition, and family history of alcoholism were measured. At baseline, depression and liver function were significant predictors of neuropsychologic performance. When tested again at 3 months, estimates of alcohol consumption and depressive symptoms were the only significant predictors.

NEUROPSYCHOLOGIC TEST PERFORMANCE BEFORE AND AFTER ORTHOTOPIC LIVER TRANSPLANTATION

Substantial improvement in neuropsychologic test performance is evident 1 year following successful liver transplantation both in alcoholics and nonalcoholics (Arria et al. 1991*c*; Tarter et al. 1990). One preliminary study testing a small group of alcoholics before and following transplant provides evidence that, except for memory capacity, the extent of improvement is substantial. Essentially, most alcoholics performed close to normal on neuropsychologic tests following transplants. These results indicate that the correction of the hepatic disease by liver transplantation in alcoholic cirrhosis is associated with substantial recovery of neurocognitive capacity.

CLINICAL RAMIFICATIONS OF HEPATIC-CEREBRAL INTERACTIONS IN ALCOHOLISM

Research into the relationships between liver and brain function in alcoholics has very important clinical ramifications. First, it underscores the potential for restoration of optimal neuropsychologic capacity following effective treatment of hepatic disease. Although the findings reviewed have been confined to a liver transplantation population, other medical interventions,

such as the administration of lactulose (a mixture of sugars used to treat ammonia intoxication), have also been reported to improve the neuropsychologic test performance of alcoholics (Conn and Lieberthal 1978). Taken together, these findings suggest that improving the methods of medical management of alcoholic liver disease may also have the benefit of improving cognitive capacity.

Second, recent findings underscore the importance of both hepatic metabolic factors and portal systemic shunting underlying hepatic encephalopathy. Besides injury and functional incapacity of the liver (Jones 1988), recent findings also suggest an important influence of nutritional factors, especially a vitamin A and E deficiency on psychomotor and visuospatial function in liver disease (see also Hunt, chapter 15). Approximately 40 percent of nonalcoholics and nonalcoholic cirrhotics have biochemical evidence of vitamin E deficiency (Arria et al. 1992). Interestingly, preliminary evidence reveals an association between the severity of vitamin E deficiency and psychomotor impairment (Arria et al. 1990*a*). Studies are currently underway to determine whether oral supplementation with vitamin E reduces these psychomotor deficits. Notably, vitamin E deficiency in children, secondary to cholestatic liver disease or diseases of malabsorption, is characterized by motor incoordination, ataxia, and neuropathy. These disturbances are reversible with vitamin E supplementation early in the course of neuromuscular degeneration (Guggenheim et al. 1982).

Third, research suggests that the susceptibility to cirrhosis is not randomly distributed in the population. Genetic factors appear to predispose individuals to cirrhosis (Hrubec and Omenn 1981). For example, a family history of alcoholism is associated with an earlier age of onset of cirrhosis (Arria et al. 1990*b*).

Furthermore, indicators of liver malfunction may appear quite early in the development of alcoholism. In one study of adolescent alcoholics undergoing treatment, up to 24 percent of those assessed had at least one biochemical indicator of liver injury (Arria et al. 1991*a*). These latter results illustrate that the onset of liver injury may occur much earlier in life than generally believed. Rapid progression to advanced liver disease has been associated with only modest levels of alcohol consumption (Cuellar et al. 1987). Also, because women are apparently more susceptible to cirrhosis than men and African Americans are more vulnerable than Caucasians, it is important to identify the factors responsible for their augmented risk for cirrhosis (see also Glenn, chapter 9). Once these factors are identified, it may then be possible to prevent cirrhosis and chronic hepatic encephalopathy.

Finally, research into hepatic-cerebral interactions has important implications for improving the identification and treatment of alcoholics. For example, alcoholics who exhibit only the medical complications of alcoholism seek treatment later in life and in more advanced stages than individuals who have experienced emotional and social problems associated with excessive drinking (Wodak et al. 1983). One possible reason for the delayed entry into treatment of

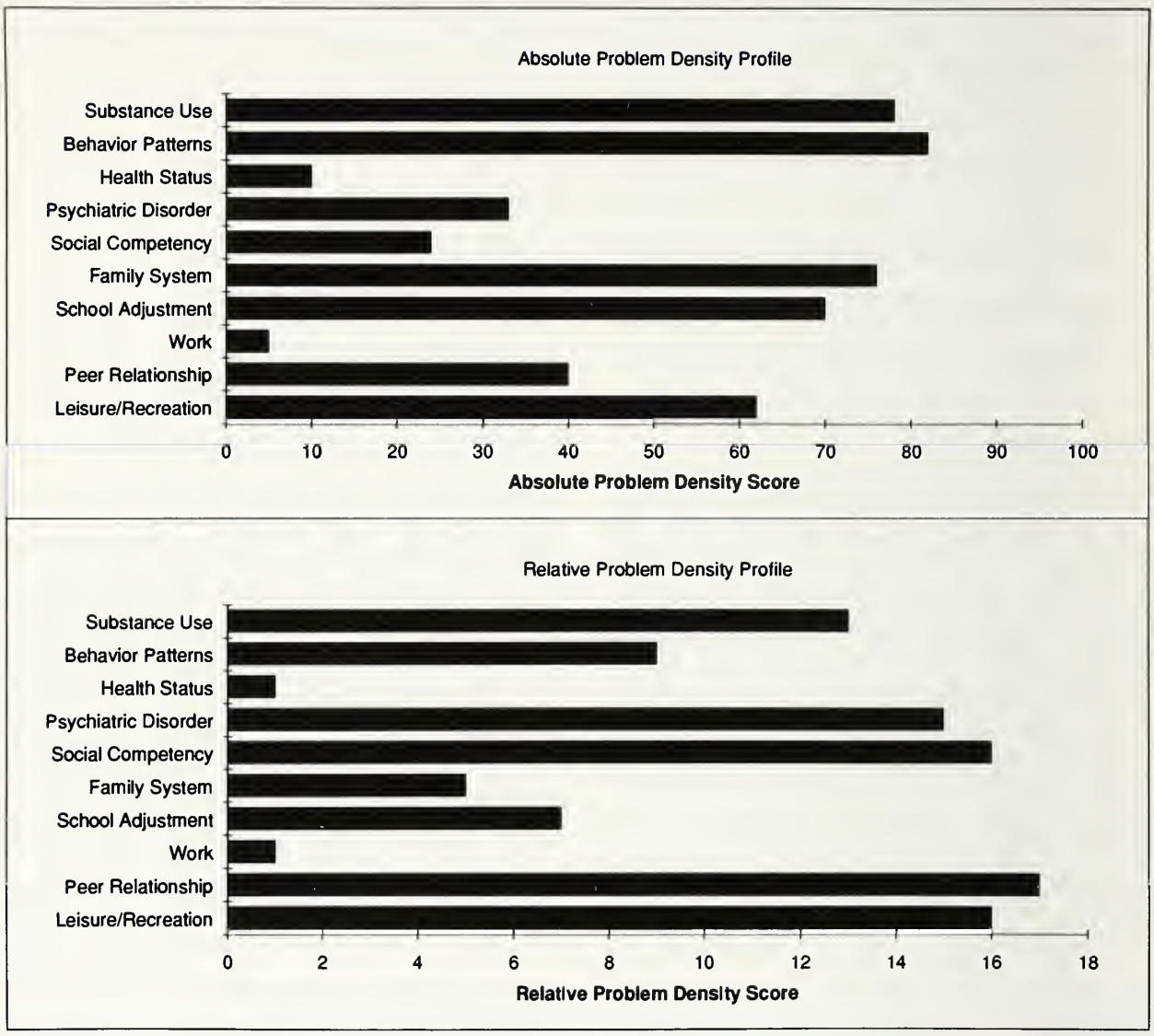

FIGURE 1

Sample profile employing the Drug Use Screening Inventory.

alcoholics having only medical problems is that the disease sequelae of alcoholism, without psychological or social disturbances, do not capture the attention of significant others, such as family members, friends, or employers, until the alcoholism is very advanced. Liver pathology, for example, typically progresses without profound symptoms or physical distress. In effect, chronic abusive drinking results in a "silent" medical deterioration until the endstage of illness, when physical discomfort from either cirrhosis or alcoholic hepatitis with cirrhosis prompts the person to seek medical care. Under these circumstances, treatment is expensive, difficult, and only rarely fully effective. An integrative approach to the problem of alcoholism, which gives equal emphasis to diagnosis of the medical, psychiatric, and psychosocial problems concomitant to chronic excessive alcohol consumption, should improve overall treatment efficacy and rehabilitation prognosis.

One step in this direction has been the recent development of the Drug Use Screening Inventory (Tarter 1990). It is useful in primary care settings, as well as

in psychiatric and forensic settings. This self-report questionnaire quantifies problems associated with alcohol and drug consumption across 10 domains. Figure 1 depicts a typical profile. The profile at the top quantifies the absolute severity of problems in each domain. This score reflects the percent of problems specific to each domain. The profile at the bottom describes the relative severity of problems across domains, yielding a total score across the 10 dimensions of 100 percent. One advantage of the Drug Use Screening Inventory is that it enables the assessor to determine at a glance the person's major problem areas, so that appropriate targeted interventions can be applied. By conceptualizing alcoholism within such a multivariate perspective that encompasses health status, psychiatric status, and psychosocial adjustment, it is then possible to implement a comprehensive rehabilitation program.

SUMMARY

Mounting evidence indicates that advanced liver disease contributes substantially to the neuropsychologic, neuroanatomic, and neurophysiologic disturbances commonly found in alcoholics. The findings show that hepatic encephalopathy frequently coexists with alcohol-induced CNS disorders in chronic alcoholics. The observation that hepatic encephalopathy is largely reversible following orthotopic liver transplantation, and possibly following aggressive medical management of the cirrhosis (particularly if recognized early), has important ramifications for the conceptualization and implementation of comprehensive rehabilitation. In the context of comprehensive multidisciplinary treatment, improvement in cognitive functioning is a major treatment objective, because optimal cognitive capacity is one component of productive social adjustment. Social adjustment, in turn, ultimately impacts positively on improving the alcoholic's overall quality of life.

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22

THE CHOLINERGIC DEAFFERENTATION OF THE CEREBRAL CORTEX INDUCED BY CHRONIC CONSUMPTION OF ALCOHOL: REVERSAL BY CHOLINERGIC DRUGS AND TRANSPLANTATION

Thomas Arendt, M.D., D.Sc.[1]

INTRODUCTION

The deleterious effects of alcohol consumption on mental abilities have been known since ancient times and are widely accepted today. Surprisingly little is known, however, about the mechanism by which alcohol damages the brain, and there is an ongoing debate on what might be the neuronal mechanism of the impaired cognitive function. Severe memory impairment is the outstanding feature of the chronic syndrome of Korsakoff's psychosis. This disorder is believed to account for the majority of alcoholics who suffer serious long-term cognitive deficits. Chronic cognitive impairment other than Korsakoff's psychosis (Lishman 1981) is still poorly defined today and will be discussed elsewhere in this book (Oscar-Berman and Hutner, chapter 6; Cermak, chapter 7; Parsons, chapter 8).

Korsakoff himself reported pathological changes at the level of the neocortex (Korsakoff and Serbski 1892), and the pathological basis of the syndrome that bears his name was almost universally regarded as cortical in location for nearly half a century (Cole 1902; Mott 1910; Carmichael and Stern 1931; Marcus 1931; Courville 1955). This view was seriously challenged by Gamper (1928), declaring that lesions in the midbrain, around the walls of the third ventricle, and in the mammillary bodies were essential, i.e., in those regions that are affected in Wernicke's encephalopathy. Eventually, a virtually exact parallel between the pathologies of Wernicke's encephalopathy and Korsakoff's psychosis was reported, largely refuting a predominately cortical basis for the disorder (Malamud and Skillicorn 1956; Victor et al. 1971). Cortical changes, however, appear to

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affect chronic alcoholics frequently (Courville 1955; Neuberger 1957; Lynch 1960; Haug 1968; Lereboullet et al. 1956). This cortical pathologic process, however, might preferentially involve a degeneration of fibers in other areas of the brain rather than cortical neurons (Lynch 1960; Cole 1902; Mott 1910), resulting in damage of intrinsic and particularly extrinsic neuronal systems.

The concept of Korsakoff-like amnesia resulting from an incomplete activation of an otherwise undisturbed cortical apparatus has already been put forward by Stertz (1931). This concept is based on the observation that a blockade of cholinergic neurotransmission involved in the mechanisms of cortical activation (McCormick and Prince 1986) produces an amnesic syndrome (Deutsch 1971). Comparative studies on the pattern of memory dysfunction induced by application of scopolamine and the deficits associated with Korsakoff's syndrome have revealed striking similarities (Kopelman and Corn 1988). However, this similarity between the neuropsychological phenomenology of pharmacological blockade of cholinergic transmission and that of alcohol abuse is not the only factor that supports the hypothesis that cholinergic neurotransmission is a primary target of

TABLE 1

Central cholinergic systems

		Target area of projection neurons
1. Local circuit neurons		
• Striatum		
• Limbic striatum (nc. accumbens, tub. olfactorium)		
• Cerebral cortex (neocortex, hippocampus)		
2. Projection neurons		
• Projection system of the basal forebrain		Cortical mantle
"Rostral cholinergic column"		
- Medial septal nucleus	Ch1	- Hippocampus
- Diagonal band nucleus	Ch2/3	- Hippocampus, olfactory bulb, limbic cortices
- Basal nucleus of Meynert	Ch4	- Neocortex, amygdala
• Projection system of the upper brain stem		Thalamus
"Caudal cholinergic column"		
- Pedunculopontine tegmental nucleus	Ch5	
- Laterodorsal tegmental nucleus	Ch6	
3. Motoneurons, neurons of cranial nerves, sympathetic and parasympathetic nervous system		

Notes: Modified after Butcher and Woolf (1986) and Satoh et al. (1983).
Nomenclature of Ch subgroups of cholinergic projection neurons after Mesulam et al. (1983a,b).

the action of alcohol on the brain (Massarelli 1979). In postmortem studies and in animal experiments, there is even more direct evidence, which will be summarized in the present chapter.

PRINCIPLES OF ORGANIZATION OF CENTRAL CHOLINERGIC SYSTEMS

Cholinergic neurons, which use acetylcholine as a transmitter at their synapses, do not form a uniform neuronal network in the brain. Several groups of neurons that form structural-functional systems can be delineated based on their connections within the brain. Table 1 summarizes the three major classes of central cholinergic neurons: (1) cholinergic interneurons, (2) cholinergic projection neurons, and (3) cholinergic neurons of cranial nerves, motoneurons, and neurons of the sympathetic and parasympathetic system. This chapter will focus on the first two classes, particularly on the cholinergic projection neurons of the basal forebrain that provide cholinergic innervation for the entire cortical mantle (Wenk et al. 1980; Mesulam et al. 1983*a*,*b*). These neurons are severely affected in a group of neuropsychiatric disorders associated with cognitive dysfunction, including postalcoholic Korsakoff's syndrome (Arendt et al. 1983).

Neurons of the nucleus basalis of Meynert (NbM), extending in the human brain ventrally to the putamen and globus pallidus from the level of the corpora mammillaria into the septum, do not form a nucleus in the classical sense. Embedded in a fiber system, the neurons are arranged in different cell clusters. Cholinergic neurons forming most of the NbM are mingled with neurons containing neurotransmitters other than acetylcholine, in particular gamma-aminobutyric acid (Brashear et al. 1986; Fisher et al. 1988), and a variety of neuropeptides (Walker et al. 1989; Senut et al. 1989), some of them colocalized with acetylcholine (Melander et al. 1985). To overcome the confusion in the literature that has arisen from the difficulty in defining the anatomical boundaries of the nuclear complex of the NbM (Arendt and Bigl 1986), an alternative "cholinergic" nomenclature was introduced by Mesulam et al. (1983*a*,*b*), which classifies cell clusters of cholinergic neurons in the basal forebrain and upper brain stem based on their cytological, topographical, and connectional properties (figure 1/table 1).

The Basal Forebrain Cholinergic System as a Component of the Ascending Reticular Activation System

Two major types of neurons, multipolar giant neurons and reticular neurons, can be discriminated based on cytological criteria in Golgi-impregnated material of the human NbM (Arendt et al. 1986). Most neurons, particularly the cholinergic neurons, are represented by the reticular type. Leontovich and Zhukova (1963) described this reticular type of neuron as the major cellular constituent of the reticular formation. Embedded in fiber systems, these neurons are arranged as discontinuous aggregations of cell clusters with overlapping dendritic trees, thus

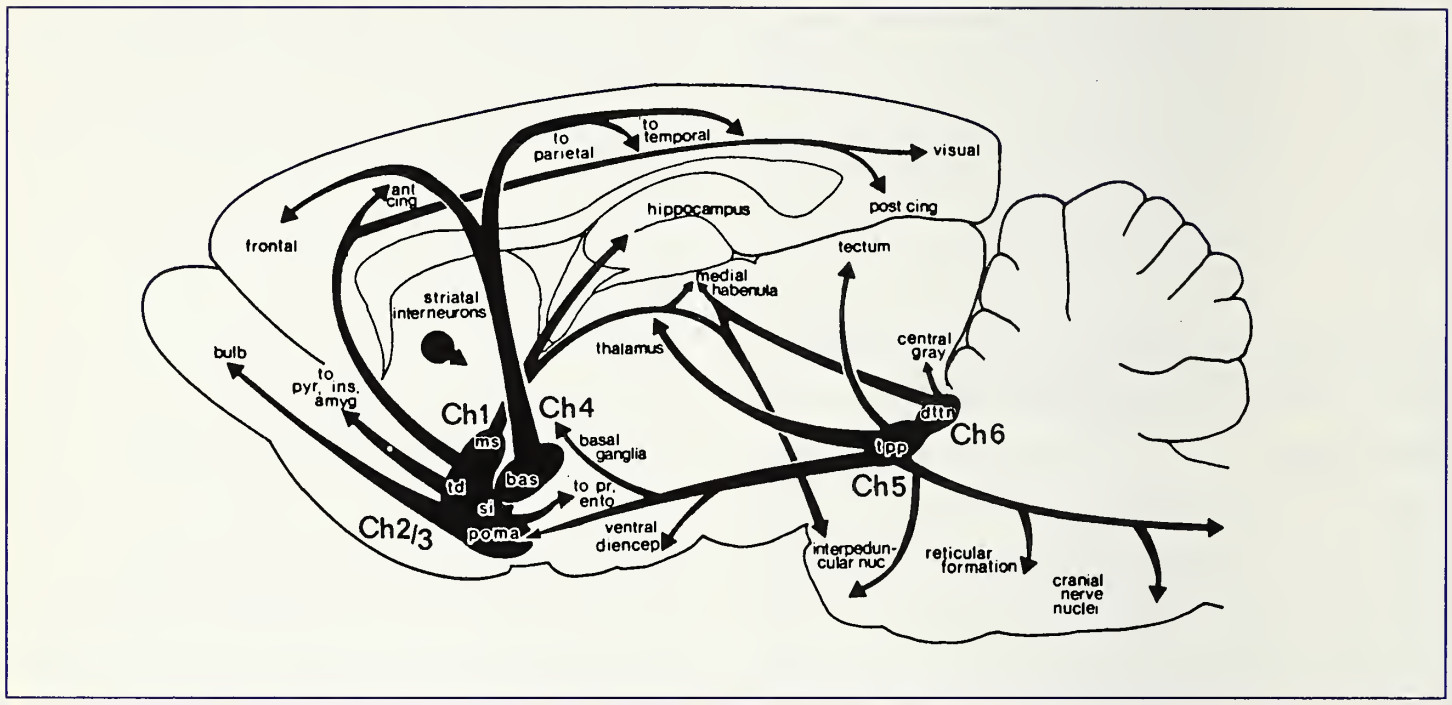

FIGURE 1

Schematic representation of the cholinergic projection systems of the basal forebrain and the upper brain stem in rat. Abbreviations Ch1 to Ch6 refer to nomenclature of cholinergic subdivisions according to Mesulam et al. (1983*b*): Ch1, Nucleus septi medialis (ms); Ch2/3, Nucleus tractus diagonalis Broca (td); Ch4, Nucleus basalis Meynert (bas), substantia innominata (si), Nucleus praeopticus magnocellularis (poma); Ch5, Nucleus tegmenti pedunculopontinus (tpp); Ch6, Nucleus laterodorsalis tegmenti (dltn). Modified with permission from Butcher and Woolf (1986).

forming a neuronal network not confined to anatomical boundaries. This network extends from the spinal cord throughout the brain axis, up to the diencephalon and telencephalon, with the NbM as its most rostral part. This concept of the "reticular core," as formulated by Leontovich and Zhukova (1963), was based on cytological features and the fine structural arrangement of the neurons. They suggested that these neurons might exert an integrative function by controlling the activity of large areas of the brain. Based on a more detailed knowledge of the chemoarchitectonic properties of the NbM, and in particular on its organizational structure, the concept of the NbM as a central integrator of limbic signals was recently developed by Wenk (1989).

The cholinergic neurons of the basal forebrain innervate the entire cortical mantle topographically. The neocortex and amygdala receive most of their cholinergic input from the NbM, whereas the hippocampus is innervated from neurons in the septum-diagonal band (table 1). The projection from the basal forebrain onto the cerebral cortex is topographically organized on an anterior-posterior as well as a ventrolateral-dorsomedial level (Bigl et al. 1982; Luiten et al. 1987; Mesulam et al. 1983*a*). In the rodent, afferent fibers of individual neurons in the NbM have restricted cortical target areas with a diameter of about 1 to 2 mm (Aston-Jones et al. 1984; Price and Stern 1983). The small projection area of individual cholinergic neurons might be about a cor-

tical macrocolumn. If true, this suggests that although the system as a whole may have a wide cortical influence, individual elements may operate independently of one another and may adjust cortical neuronal excitability on a module-by-module basis (Wenk 1989).

Anatomical, electrophysiological, and pharmacological properties of the cholinergic NbM neurons and their effects on cortical excitability indicate that this system might be used for setting the tone for cortical processing (Steriade and Buzsaki 1990). The ability of acetylcholine to reduce the resting potassium current, besides the voltage- and calcium-dependent potassium currents, enables the cholinergic projection neurons to enhance the excitability of cortical neurons by sensory inputs. In addition to this excitatory effect, acetylcholine has also been reported to cause an inhibition mediated by the excitation of local GABAergic interneurons (McCormick 1990). This capability might give the ascending cholinergic system additional control over the excitability of the cerebral cortex by altering the spread and synaptic course of information flow.

The relay nuclei of the thalamus receive approximately 80 percent of their cholinergic input from the projection system of the upper brain stem (Mesulam et al. 1983*b*). The nucleus reticularis thalami receives a dual cholinergic innervation

TABLE 2

Pattern of neuropsychological dysfunction in mental disorders and after "blockade" of cholinergic synapses in young healthy volunteers by scopolamine

	Scopolamine	Korsakoff's syndrome	Alzheimer's disease	Huntington's chorea
Cholinergic afferentation of the cortical mantle		↓	↓	–
Memory				
Primary memory	–	–	↓	–
Secondary anterograde memory				
Explicit component				
Semantic memory	↓	↓	↓	–
Spatial memory	↓	↓	↓	–
Implicit component				
Procedural and skill learning	–	–	–	↓
Priming	–	–	–	↓
Secondary retrograde memory	–	–	–/↓	↓
Conceptual ability and behavioral regulation	↓	↓	↓	–
Attention	↓	–	↓	↓

Notes: ↓ *impaired;* – *no impairment.*
Data adapted from Brown and Marsden (1988) and Kopelman and Corn (1988).

from both the upper brain stem and a subpopulation of cortical projection neurons in the basal forebrain (Jourdain et al. 1989). Since the nucleus reticular thalami might be the pacemaker for the generation of certain types of rhythmic activity in the brain, this anatomical arrangement might have important implications for the cholinergic control of thalamocortical processing.

Stimulation of the mesencephalic reticular formation that leads to desynchronization of the electroencephalogram (EEG) also leads to dramatic increases in the release of acetylcholine in the cerebral cortex. Similarly, the rate of release of acetylcholine in the cerebral cortex is correlated with the level of arousal (see McCormick 1990).

Thus, the cerebral cortex is activated by a dual mechanism, the direct influence of cholinergic afferentation from the basal forebrain and the suppression of pacemaker cells in the nucleus reticularis thalami (Buzsaki et al. 1988). The cholinergic projection systems of both the basal forebrain and the upper brain stem, designated as the "rostral" and "caudal cholinergic column" (Satoh et al. 1983), can therefore be viewed as the anatomical substrate of the ascending activation system described by Moruzzi and Magoun (1949). The hypothesis presented in this present paper suggests that mental dysfunction observed after both acute and chronic ethanol consumption can largely be attributed to an impairment of the cholinergic pathway of the ascending activation system, appearing either acutely as an "anticholinergic syndrome" or chronically as a "syndrome of partial cholinergic deafferentation of the cortical mantle."

THE INHIBITORY ACTION OF ETHANOL ON CHOLINERGIC NEUROTRANSMISSION: THE ACUTE PHARMACOLOGICAL BLOCKADE

Neuropsychology

Identification of both common and specific steps in the pathogenesis of different forms of dementia and investigation of the relationship between these events and cognitive disturbances may help to define the neurobiological substrate of these disorders and may suggest approaches for their management.

Comparative neuropsychological studies clearly indicate that, in some respects, early Alzheimer's disease and postalcoholic Korsakoff's syndrome show similar patterns of memory impairment, but are differentiated from the memory impairment in Huntington's disease (Butters 1985; Butters et al. 1983; Moss et al. 1986) (table 2). The similarities between Alzheimer's disease and Korsakoff's syndrome might be restricted to the initial memory dysfunction. Beyond the very early stages, patients with Alzheimer's disease develop an additional disruption of language and constructional abilities, which probably relates to more widespread cerebral damage. This type of disruption is in contrast to the amnesia in Korsakoff's syndrome. However, after a long drinking history, some severe alcoholics develop intellectual impairments

that exceed those with Korsakoff's syndrome and resemble the clinical picture of Alzheimer's disease (Lishman 1986; King 1986). Moreover, there are indications that many patients with Korsakoff's syndrome may have cognitive dysfunctions extending beyond memory deficits and should be regarded as demented (Lishman 1986). It has been pointed out that an unknown number of alcohol abusers suffering from alcoholic dementia might be misdiagnosed as having Alzheimer's disease (King 1986; also see Dufour, chapter 1). This difficulty in establishing alcoholic dementia as a distinct disorder and distinguishing it from Alzheimer's disease might be explained by a common neurobiological substrate underlying both alcohol-induced brain damage and Alzheimer's disease.

The anterograde amnesic deficit described for Alzheimer's disease and alcoholic Korsakoff's syndrome most likely reflects primarily an acquisition or learning deficit. In this respect, the deficit resembles the amnesia induced by the application of anticholinergic drugs to healthy subjects (Kopelman 1985). Particularly on tests of secondary memory, "cholinergic blockade" by scopolamine produces a pattern similar to that seen in both disorders. The characteristics include a pronounced impairment in learning verbal and visuospatial material,

TABLE 3

The central depressant action of ethanol:
Pharmacological evidence for an involvement of cholinergic neurotransmission

Physostigmine counteracts of the following effects of ethanol:

In rodents:

- •Depressant effects on visually evoked potentials (Hetzler and Smith 1984)
- •Sleep-time (Erickson and Burnam 1971)
- •Cortical EEG synchrony (Erickson and Chai 1976)
- •Memory impairment (Beracochea et al. 1986; Arendt et al. 1990)

In humans:

- •Alcohol delirium and intoxication (Daunderer 1978; Rupreht et al. 1989)
- •Amnesia (Nabeshima et al. 1991) and impairment in verbal learning (Hrbek et al. 1986)

Nicotine counteracts the following effects of ethanol:

In humans:

- •Increased reaction time (Myrsten and Anderson 1975)
- •Impairment in visual discrimination, choice reaction time, time judgement, auditory attention (Holloway and Holloway 1979)
- •Slowing of alpha activity of EEG (Knott and Venables 1979)

Oxotremorine counteracts the following effects of ethanol:

In rodents:

- •Impairment of retention of conditioned stimulus (Brioni et al. 1989)

TABLE 4

Effects of ethanol administration on central cholinergic systems

Kind of application		Species	Brain region	Reference
Acetylcholine concentration				
Acute	↑	Rat/mice/rabbit	Cerebral cortex	Erickson and Graham 1973
			Caudate nucleus	Hunt and Dalton 1976
			Brain stem	Parker et al. 1978
	↓	Mice	Whole brain	Rawat 1974
Chronic	↓	Rat/mice	Cerebral cortex	Arendt et al. 1989*a*
			Hippocampus	Hunt and Dalton 1976
			Basal forebrain	Moss et al. 1967
			Caudate nucleus	Rawat 1974
			Brain stem	Smyth and Beck 1969
Acetylcholine release				
In vitro				
Acute	↓	Rat	Cerebral cortex	Kalant et al. 1967
				Kalant and Grose 1967
				Carmichael and Israel 1975
Chronic	↓	Rat	Cerebral cortex	Arendt et al. 1989*a*
			Hippocampus	
			Basal forebrain	
In vivo				
Acute	↓	Cat/rabbit	Cerebral cortex	Erickson and Graham 1973
			Reticular formation	Morgan and Phillis 1975
				Phillis and Jhamandas 1971
Acetyl-CoA availability (AcetylCoA content/pyruvate dehydrogenase activity)				
Acute	↓	Mice	Whole brain	Rawat 1974
Chronic	↓	Rat/mice	Cerebral cortex	Arendt et al. 1989*a*
			Hippocampus	Rawat 1974
			Basal forebrain	Smyth et al. 1967
				Smyth and Beck 1969
Choline uptake				
In vitro				
Acute	↓	Synaptsomes/rat	Forebrain	Mrak and North 1988
Chronic	↑	Glioblast/neuroblast cell lines		Massarelli et al. 1976
In vivo				
Acute	↑	Rat	Caudate nucleus	Hunt et al. 1979
	↓	Rat/mice	Caudate nucleus	Durkin et al. 1982
			Hippocampus	Hunt et al. 1979
Chronic	↓	Rat	Cerebral cortex	Arendt et al. 1989*a*
			Hippocampus	Beracochea et al. 1986

TABLE 4 *CONT'D*

Effects of ethanol administration on central cholinergic systems

Kind of application		Species	Brain region	Reference
Acetylcholine synthesis/choline acetyltransferase activity				
Acute	↑	Mice	Cortex	Soliman and Gabriel 1985
			Hippocampus	Durkin et al. 1982
			Caudate nucleus	
	↓	Mice	Cerebral cortex	Owasoyo and Iramain 1981
			Hippocampus	Rawat 1974
Chronic	↓	Rat/mice	Cerebral cortex	Arendt et al. 1989*a*
			Hippocampus	Pelham et al. 1980
			Hypothalamus	Rawat 1974
			Caudate nucleus	Smyth et al. 1967
			Basal forebrain	Smyth and Beck 1969
Acetylcholinesterase activity				
Acute	↑	Mice	Cortex	Soliman and Gabriel 1985
			Hippocampus	
	↓	Mice	Cerebral cortex	Owasoyo and Iramain 1981
Chronic	↓	Rat/mice	Cerebral cortex	Arendt et al. 1989*a*
			Hippocampus	Kinard and Hay 1960
			Caudate nucleus	Rawat 1974
			Basal forebrain	Smyth and Beck 1969
			Brain stem	Smyth et al. 1967
Muscarinic receptors				
Chronic	↑	Rat/mice	Cerebral cortex	Hoffman et al. 1986
			Hippocampus	Muller et al. 1980
			Caudate nucleus	Pelham et al. 1980
			Forebrain	Pietrzak et al. 1990
				Rabin et al. 1980
				Smith 1983
				Smyth and Beck 1969
				Tabakoff et al. 1979

Note: ↓ *decreased;* ↑ *increased.*

a "normal" forgetting rate once learning was accomplished, and relative preservation of the response to priming and of skill learning (procedural memory) (table 2).

Clinically, this central pharmacological blockade of cholinergic neurotransmission appears as a syndrome known as the "central anticholinergic syndrome" (Schneck and Rupreht 1989). Behaviorally, the syndrome is characterized by agitation, seizures, restlessness, hallucinations, disorientation, depression, stupor, coma, and respiratory depression. The anaesthesia and intensive care litera-

ture dating from the beginning of the century report an amnesic effect of anticholinergic drugs, which were frequently used as a premedication for surgical operation (Gauss 1906; Thompson and Cotterill 1909; Pandit and Dundee 1970). Physostigmine prevents the central anticholinergic syndrome (Schneck and Rupreht 1989). Because physostigmine is effective in the clinical management of several effects of alcohol, especially alcohol delirium and stupor (Daunderer 1978; Rupreht et al. 1989), one might assume that an anticholinergic syndrome is part of the clinical picture of acute alcohol intoxication. Table 3 summarizes some evidence describing several sequelae of acute as well as chronic alcohol consumption, largely mediated by a depressant action on cholinergic neurotransmission.

Neurochemistry

Consumption of ethanol affects a variety of neurotransmitter functions in the brain (Hunt and Majchrowicz 1979; Shanley and Wilce, chapter 14). The most consistent data, however, have been obtained on the action of ethanol on cholinergic neurotransmission (Massarelli 1979), which are summarized in tables 4 and 5. The release of acetylcholine is most sensitive to the inhibitory action of ethanol, as compared with the release of serotonin, dopamine, noradrenaline, glutamate, and gamma-aminobutyric acid, suggesting a direct effect of ethanol on cholinergic neurons (Carmichael and Israel 1975). The effects of ethanol on acetylcholine release are parallelled by its behavioral effects and by an ethanol-induced EEG synchrony, the latter being regarded as an indicator of the central nervous system depression (Sauerland and Harper 1970; Erickson and Graham 1973). Furthermore, the observation that the mesencephalic reticular formation appears even more sensitive to the depressant actions of ethanol on acetylcholine release than the cerebral cortex (Erickson and Graham 1973) suggests that the central actions of ethanol are largely mediated by a depression of the cholinergic pathway of the ascending reticular activating system.

Administration of ethanol appears to affect not only the release of acetylcholine but also several steps of its metabolism (see table 4). An initial rise in acetylcholine concentrations during consumption of ethanol is followed by a decrease thereafter. Discrepancies in different studies of acute administration, as well as the differences reported for acute and chronic ethanol intake, might largely be explained by the biphasic effect of ethanol on acetylcholine metabolism (Hunt and Dalton 1976).

A progressive decrease in the synthesis, content, and release of acetylcholine as a function of the duration of ethanol intake has been demonstrated in a long-term followup study in rats (Arendt et al. 1989*a*) (figure 2). Ethanol-induced changes seem fully reversible after ethanol elimination from the blood during early treatment. After continuation of ethanol application for a longer period, however, these changes are only partially reversible or even irreversible, independent of the presence of ethanol in the blood. Thus, there may be a critical moment when the

TABLE 5

The cholinergic afferentation of the cortical mantle in chronic alcoholics

Parameter		Brain region	Reference
ChAT	↓	Cerebral cortex Hippocampus	Antuono et al. 1980 Arendt et al. 1990 Carlsson et al. 1980 Nordberg et al. 1980 Smith et al. 1988
Muscarinic receptors	↓	Cerebral cortex Hippocampus	Freund and Ballinger 1988 Freund and Ballinger 1989 Nordberg et al. 1980 Nordberg et al. 1983

Note: ↓ decreased; ChAT, choline acetyltransferase.

"pharmacological blockade" by ethanol progresses to a "neurotoxic lesion."

The sensitivity of central cholinergic neurons to ethanol appears to be different among various strains of animals, suggesting a genetic influence on the response (Durkin et al. 1982; Kochar and Erickson 1986; Fuhrmann et al. 1986; Erwin et al. 1988; Hashemzadeh and Mandel 1989; Overstreet et al. 1990). Furthermore, it is important to note that susceptibility to the deleterious effects of ethanol might differ depending on the maturity and age of an organism (Meyer et al. 1984; Bond 1986; Lancaster et al. 1986). Ethanol-induced developmental deficiencies of the central nervous system might also involve the central cholinergic system (Bond 1986; Lancaster et al. 1986; Riley et al. 1986; Light et al. 1989*a*,*b*; Okonmah et al. 1989; Brodie and Vernadakis 1990). Particularly involved may be the basal forebrain nuclei that provide the cholinergic afferentation to the hippocampus and cortex (Sulik et al. 1984; Serbus et al. 1986; Kelly et al. 1989; Schambra et al. 1990).

DEGENERATION OF CHOLINERGIC NEURONS INDUCED BY PROLONGED ETHANOL INTAKE: THE CHRONIC NEUROTOXIC LESION

Postmortem Studies

The chronic intake of ethanol in humans (Arendt et al. 1983; 1989*b*) and rodents (Arendt et al. 1988*a*,*b*, 1989*a*; Beracochea et al. 1987) results in degeneration of the cholinergic basal forebrain projection system. This degeneration is revealed by a decrease in neuronal number in the NbM and a depletion of cortical choline acetyltransferase (ChAT) activity accompanied by an impairment in memory function (see figures 2 through 6). ChAT activity is most severely affected in Korsakoff's psy-

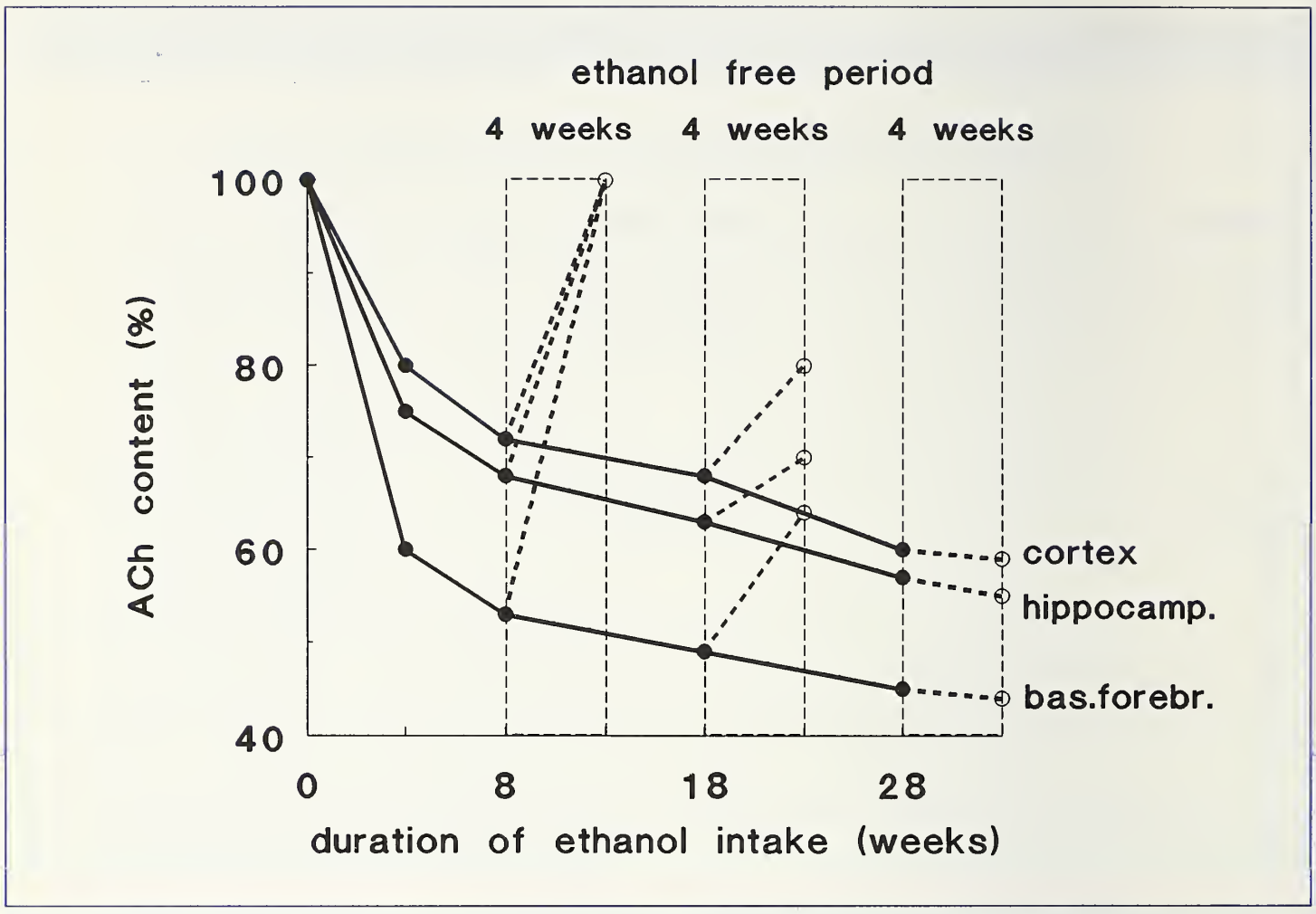

FIGURE 2

Changes in acetylcholine (ACh) content in cortex, hippocampus, and basal forebrain over the duration of ethanol intake. Content of ACh was measured after 4, 8, 18, and 28 weeks of intake of ethanol (20 percent solution in the drinking water) (black circles) and after a 4-week ethanol-free period following previous 8, 18, and 28 weeks of ethanol treatment (open circles, dotted line). ACh content shows a time-dependent decrease in all areas investigated that is fully, partially, or not reversible, depending on the duration of previous ethanol intake. Modified with permission from Arendt et al. (1989*a*).

chosis, specifically in those areas that form part of the basal forebrain cholinergic projection system and its respective target areas. However, neurodegenerative changes in this condition are more widespread and not restricted to central cholinergic systems (figure 3).

Neuronal loss in the NbM in chronic alcoholism is most pronounced in cases with Wernicke's encephalopathy, reaching about 40 to 50 percent. Cases without Wernicke's encephalopathy are less severely affected, being only marginally different from normal elderly (figure 4) (also see Harper and Kril, chapter 3). In comparison, normal aging is related to a loss of about 20 percent of neurons without being accompanied by mental impairment. On the other hand, dementia of the Alzheimer type is characterized by a loss of more than 50 percent of cells in cases with late onset and up to 80 percent in cases with early onset (figure 4). Thus, a continuum of degenerative changes occur because of aging, chronic alcohol abuse, and Alzheimer's disease.

Degeneration of cholinergic neurons due to aging, Korsakoff's psychosis, and Alzheimer's disease is not evenly distributed throughout the rostrocaudal extension of the basal forebrain projection system. Detailed morphometric investigations of different neuronal clusters of the NbM have established a degeneration pattern in cases of Korsakoff's syndrome, with Wernicke's encephalopathy being most pronounced for the most rostral parts of the cholinergic basal forebrain projection system. However, the cholinergic projection system of the upper brain stem is spared (figure 5). A similar rostrocaudal gradient in the pattern of degeneration has been obtained for the normal aging process as well as for Alzheimer's disease (Arendt and Bigl 1991). A major difference exists between Korsakoff's syndrome and Alzheimer's disease, however, with respect to the involvement of the cholinergic projection system of the upper brain stem (Ch5 and Ch6, according to the nomenclature of Mesulam et al. 1983*a*) (figures 1 and 5).

Animal Studies

The degeneration of the cholinergic afferentation of the cerebral cortex induced by chronic ethanol consumption in humans can be mimicked in rodents.

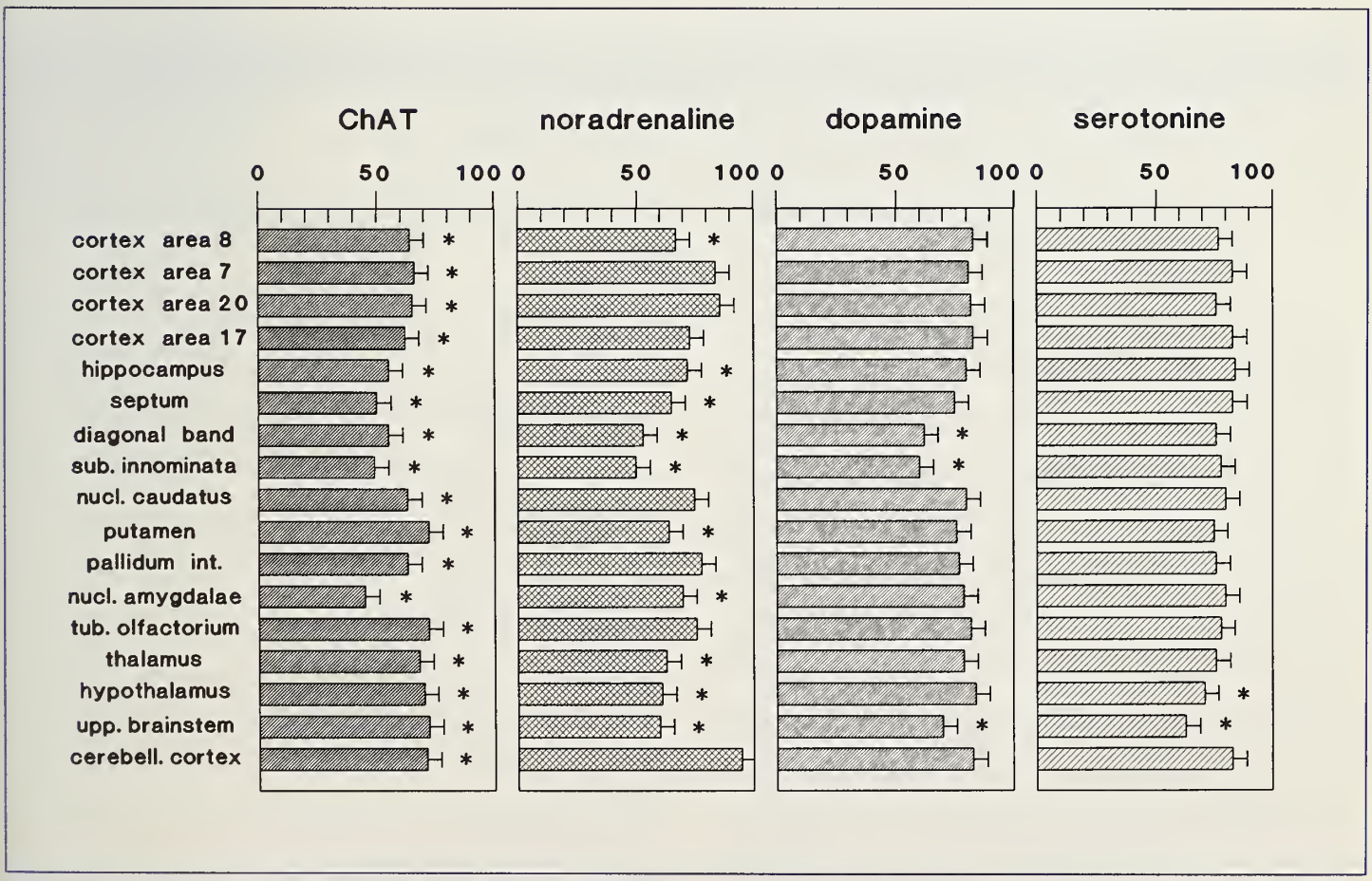

FIGURE 3

Changes in the activity of ChAT and in the content of noradrenaline, dopamine, and serotonin in postalcoholic Korsakoff's syndrome. Data are mean (± SEM) obtained on $n = 6$ cases with Wernicke's encephalopathy (mean age ± SD: 48.2 ± 4.3 years), expressed as a percentage of control values ($n = 10$, mean age: 51.6 ± 7.3). Mean postmortem delay: 3.6 ± 0.8 hours. *Differences are significant for $p < 0.05$, Student's t-test.

Consumption by rats of a 20-percent ethanol solution results in a time-dependent degeneration of the basal forebrain cholinergic projection system. This is revealed by a progressive cell loss in the NbM, parallelled by deficiencies in synthesis, content, and release of acetylcholine in target structures such as the cerebral cortex (Arendt et al. 1987, 1989*a*) (figure 6). The degeneration is accompanied by a progressive impairment in memory function, especially when the cell loss in the basal forebrain occurs as a result of irreversible changes. In the present paradigm, this occurs after about 18 weeks of ethanol intake (see figures 2 and 6).

Similar to the human brain, degeneration in the rat brain is most pronounced in the rostral parts of the cholinergic basal forebrain complex, i.e., the septal-diagonal band region; whereas the cholinergic brain stem nuclei seem unaffected (figure 7). These regional differences in the degeneration pattern might be due to differences in the regional tissue concentrations of ethanol in the brain (Erickson 1976). Concentrations are particularly high in the septal area after chronic ethanol administration (Friedman 1974). The equally substantial and progressive effects of ethanol on aminergic neurons, such as the noradrenergic locus coeruleus and the serotonergic raphe nuclei, however, precludes drawing a causal relationship between cholinergic involvement and memory dysfunction. During cortical activation, locus coeruleus and raphe neurons may have synergistic effects with the cholinergic system (Vanderwolf 1988). In particular, a role for noradrenergic neurons in learning and memory has been proposed based on both animal experiments (Crow 1973) and observations of lowered plasma levels of noradrenaline metabolites in Korsakoff amnesiacs (McEntee et al. 1984). This issue has been investigated further in several animal experiments using pharmacological intervention in cholinergic neurotransmission as well as using the technique of grafting neurons and glial cells.

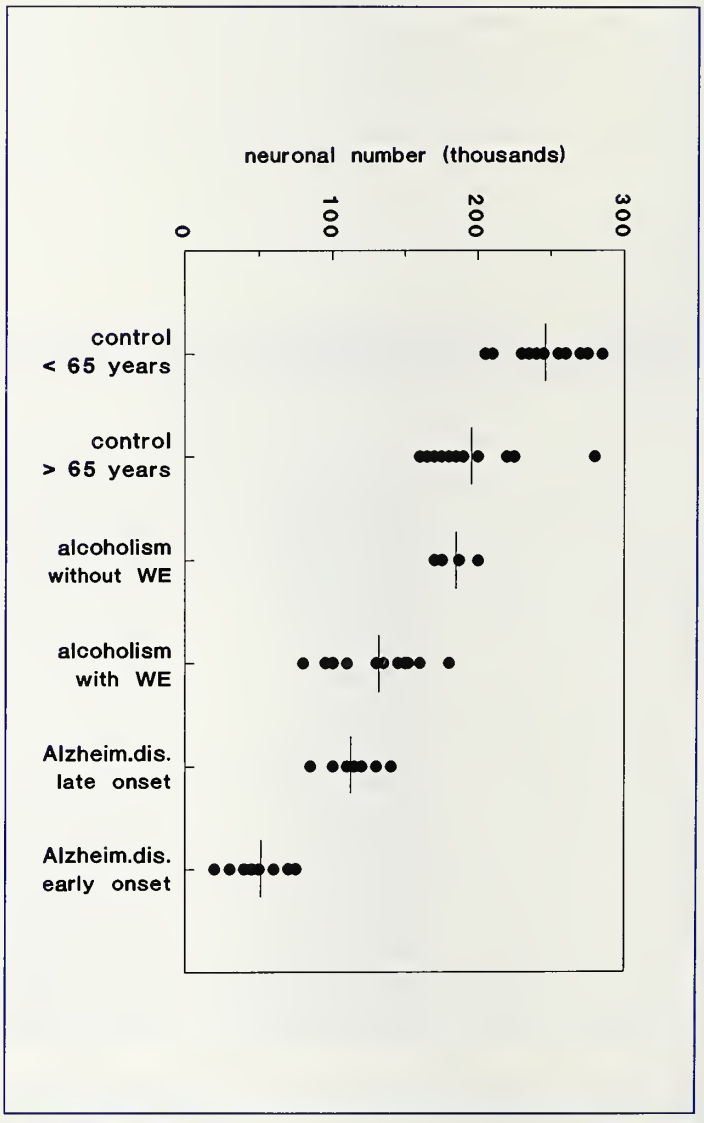

FIGURE 4

Changes in total number of neurons in the cholinergic basal forebrain projection system (Ch1 to Ch4) of 1 hemisphere in normal aging, chronic alcoholism, and Alzheimer's disease. Data indicate individual cases and the mean of each group.

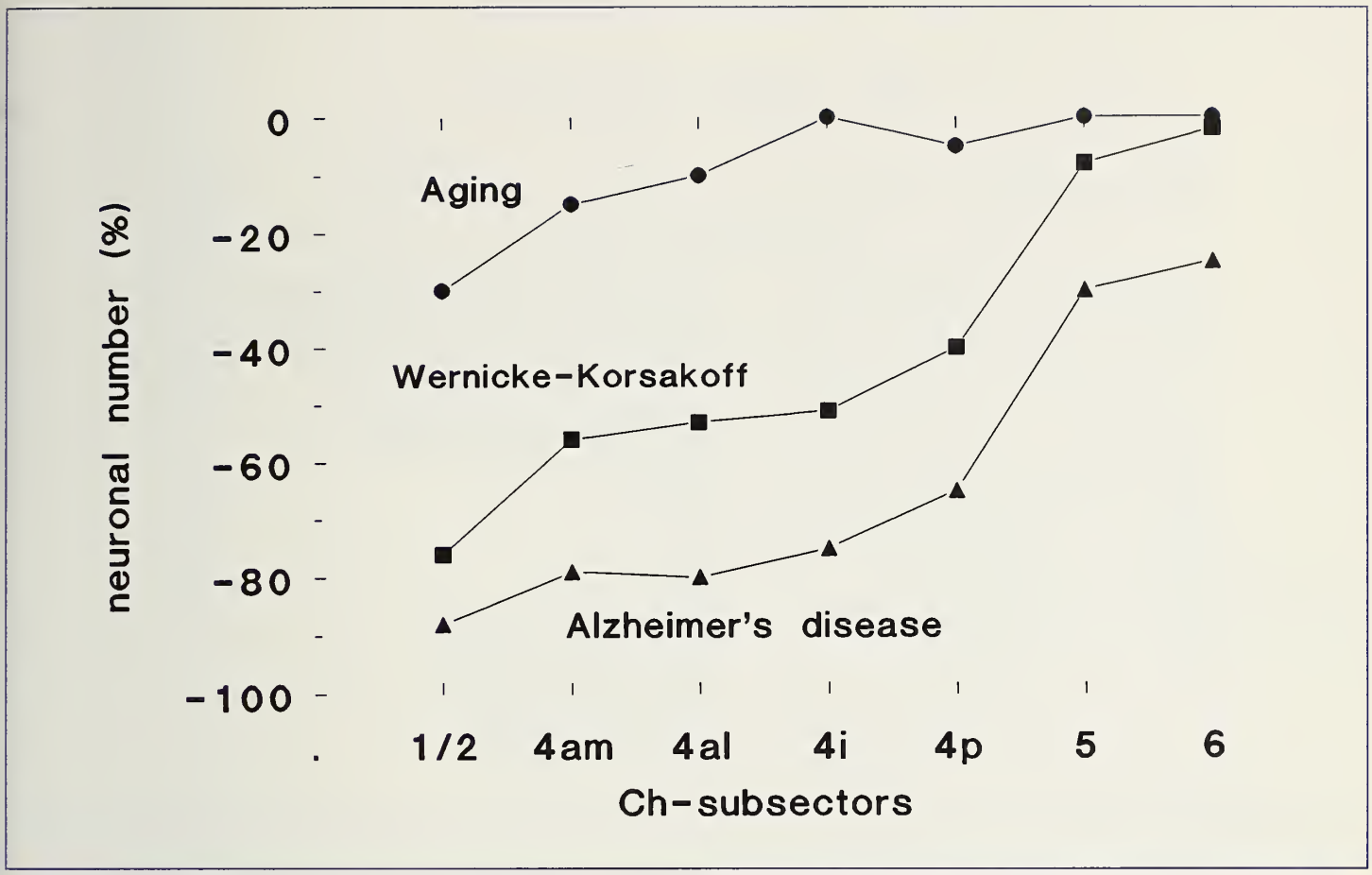

FIGURE 5

Rostrocaudal gradients in the degeneration pattern in the cholinergic projection systems of the basal forebrain and upper brain stem in normal aging, Wernicke-Korsakoff's syndrome, and Alzheimer's disease (early onset), as compared to controls younger than 65 years of age. Data are mean of 8 individuals.

Pharmacological Intervention in Cholinergic Neurotransmission After Chronic Ethanol Intake

Ethanol-induced impairment of memory function can be ameliorated by pharmacological manipulation of cholinergic neurotransmission (Arendt et al. 1990; Beracochea et al. 1986; Hodges et al. 1991; also see Martin and Nimmerrichter, chapter 23). The inhibition of acetylcholine degradation by physostigmine or the application of arecoline or nicotine as direct muscarinic and nicotinic receptor agonists are equally effective in partially restoring ethanol-induced memory deficits. However, application of neostigmine appears behaviorally ineffective (figure 8). These results agree with other studies reporting the effects of physostigmine on behavioral impairment in rodents and humans induced by ethanol consumption (Beracochea et al. 1986; Erickson and Chai 1976; Erickson and Burnam 1971; Daunderer 1978; Laurent et al. 1981).

Ethanol-induced changes, however, are not restricted to cholinergic neurons of the basal forebrain. Cholinergic interneurons, such as those of the striatum, are involved to a similar extent (figures 3 and 7) (Arendt et al. 1990; Pelham et al. 1980), which might contribute to the

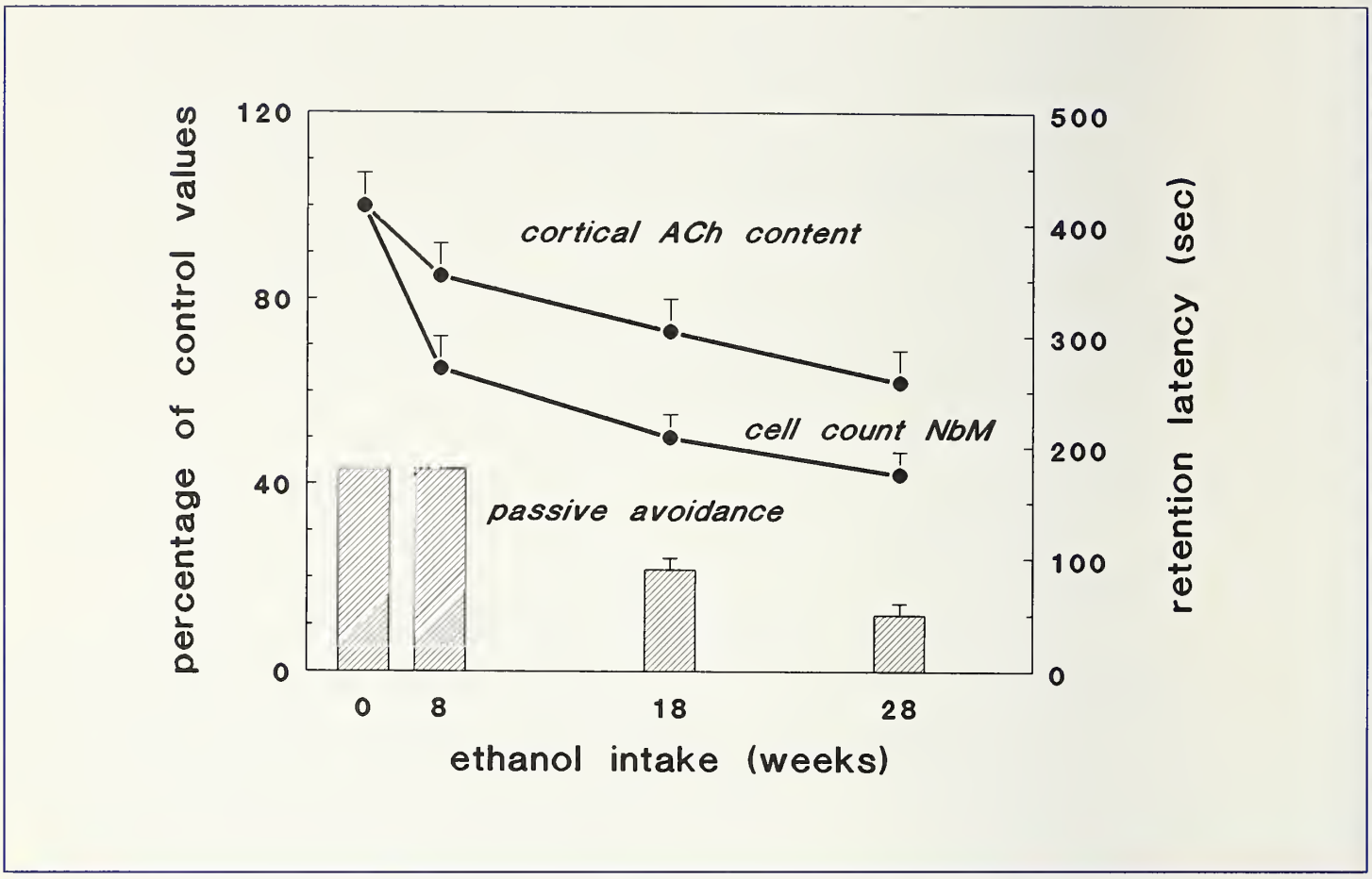

FIGURE 6

Degeneration in the basal forebrain cholinergic projection system and memory impairment as a function of the duration of ethanol intake in rat. Animals treated with ethanol (20 percent solution in the drinking water) for 18 or 28 weeks showed an impairment in the retention of a passive avoidance task (right scale, columns). The content of ACh in the frontal cortex and the neuronal number in the NbM, determined after completion of the behavioral task, showed a time-dependent decline over the duration of ethanol (left scale). Changes are expressed as a percentage of control values (+ SD) obtained on non-ethanol-treated animals (each group consisted of 12 animals).

behavioral impairment. Therefore, to establish a conclusive link between an ethanol-induced deficiency of the cholinergic afferentation of the cortical mantle and memory dysfunction, an approach is required that can more directly enhance cholinergic input to the cortex.

Transplantation of Neurons and Glial Cells

Intracortical grafting of rat fetal brain tissue derived from the primordial basal forebrain has been shown to reduce cognitive deficits caused by aging (Gage et al. 1984), excitotoxic lesions of the basal forebrain (Dunnett et al. 1985), or chronic ethanol consumption (Arendt et al. 1988*a*). As such grafts necessarily contain a multiplicity of cellular and noncellular constituents, it might be difficult to discover the critical factors underlying restoration of function. One approach compares grafts of different origins and/or types, of which some contain but others lack potentially relevant factors. In some experiments (Arendt et al. 1988*a*, 1989*a*; Brückner and Arendt 1991, 1992), we have applied this approach to investi-

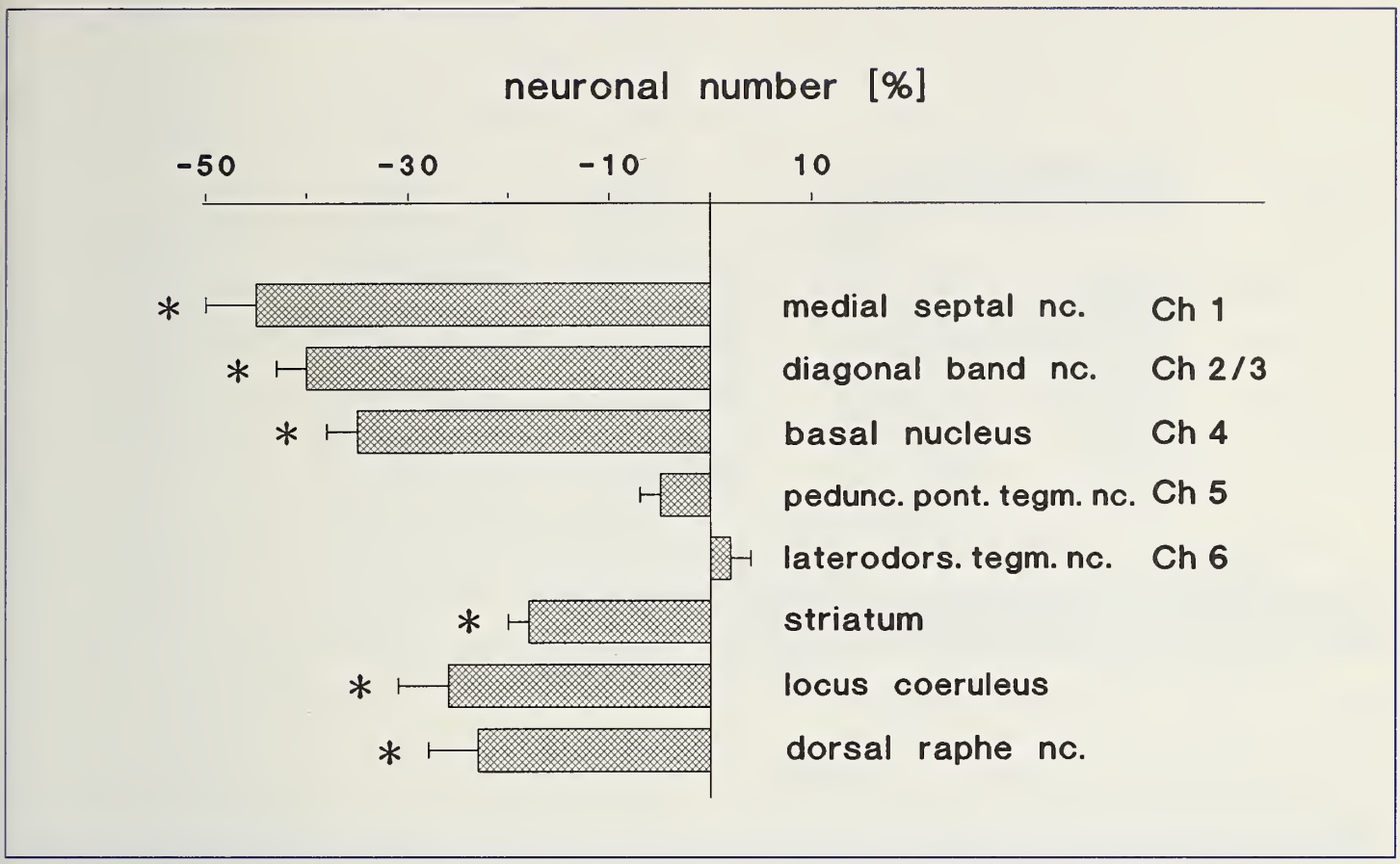

FIGURE 7

Changes in the number of acetylcholinesterase-positive neurons in the cholinergic projection systems of basal forebrain (Ch1 to Ch4) and upper brain stem (Ch5 and Ch6), the striatum, the locus coeruleus, and the dorsal raphe nucleus in rat treated with ethanol (20 percent) for 28 weeks. Data represent the mean of 8 animals, expressed as a percentage of control values (± SD). * Differences to non-ethanol-treated controls are statistically significant, $p < 0.01$ (Student's t-test).

gating the relationship between cholinergic deafferentation of the cortical mantle after chronic ethanol intake and cognitive dysfunction.

Tissue taken at embryonic day 16, either from the primordial basal forebrain or from the hippocampus, was injected into the cerebral cortex, hippocampus, or both locations of rats previously treated with ethanol.[2] Animals receiving "cholinergic" transplants improved their performance in the radial arm maze, whereas those receiving "noncholinergic" transplants remained impaired. Improvement was significant for both reference and working memory (figure 9). Behavioral recovery was dependent on the site of transplantation. It was most pronounced for the simultaneous transplantation at neocortical and hippocampal sites (figure 9).

Improvement in memory function induced by cholinergic transplants derived from the basal forebrain may result from the supply of acetylcholine by the graft or from another factor associated with cholinergic neurons. A significant

[2] *Tissue taken from the basal forebrain is rich in cholinergic neurons, whereas that from the hippocampus is largely devoid of cholinergic neurons. Consequently, grafting of the former is designated in the following discussion as "cholinergic" transplants and grafting of the latter as "noncholinergic" transplants.*

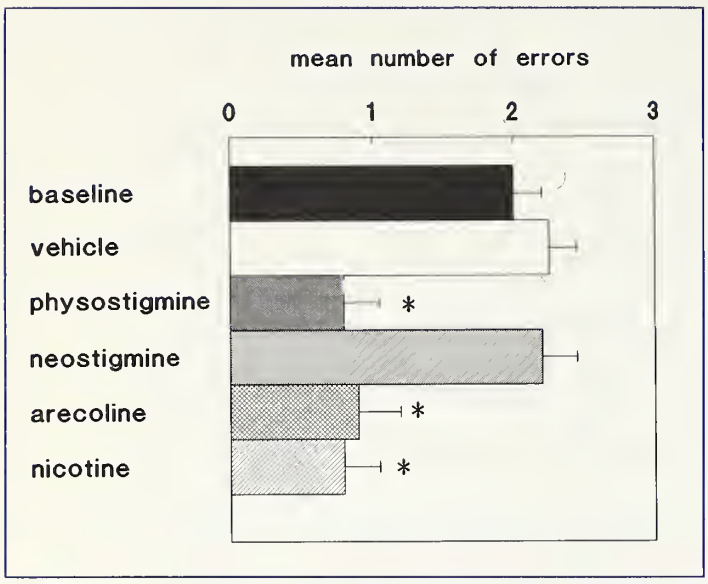

FIGURE 8

Effects of pharmacological manipulation of cholinergic neurotransmission on the asymptotic performance in the radial arm maze of rats treated with ethanol (20 percent) for 28 weeks. Drugs were applied (i.p.) 15 minutes before testing at the following dosages (per kg body weight): physostigmine 0.45 mg, neostigmine 0.45 mg, arecoline 1.00 mg, nicotine 0.10 mg. Data are mean (± SD) of 12 animals. *Differences are statistically significant $p < 0.01$ (Student's t-test).

increase in the synthesis, content, and release of acetylcholine could indeed be established at the site of implantation of tissue taken from the basal forebrain (Arendt et al. 1989*a*). Behavioral improvement after transplantation was quantitatively related to the increase in acetylcholine content but not to any changes in noradrenaline, dopamine, or serotonin content (figure 10).

Furthermore, a significant relationship was detected between the volume of cholinergic grafts and the extent of postoperative behavioral recovery (table 6). After injecting cholinergic transplants into the cerebral cortex, a recovery in synthesis and release of acetylcholine, previously reduced by chronic ethanol treatment, was also detected in the basal forebrain (Arendt et al. 1989*a*). This observation probably indicates that graft-induced behavioral recovery might be at least partially due to trophic mechanisms rather than to the formation of synapses between graft and host.

Trophic factors, which could induce behavioral recovery, appear largely derived from reactive astrocytes. Therefore, we have investigated the effects of intracortical and intrahippocampal grafting of purified astrocytes on impaired memory function induced by chronic intake of ethanol (Brückner and Arendt 1992). The results,

TABLE 6

Correlations between mean transplant and pre- and posttreatment differences in maze performance

	Volume (± SEM) in mm^3	Correlation coefficients Place task	Cue task
"Cholinergic" transplants	7.58 ± 1.32	0.95[1]	0.87[2]
"Noncholinergic" transplants	11.96 ± 5.53	0.10	0.04

Notes: The rank correlation coefficient was calculated from the difference between asymptotic performance of 5 animals, 13 weeks after receiving either cholinergic or noncholinergic transplants to cortex and/or hippocampus and their asymptotic maze performance prior to transplantation. The volume of the transplant was determined by planimetry. [1]$p < 0.01$; [2]$p < 0.05$ (Mann-Whitney u test). Modified after Arendt et al. (1989*a*).

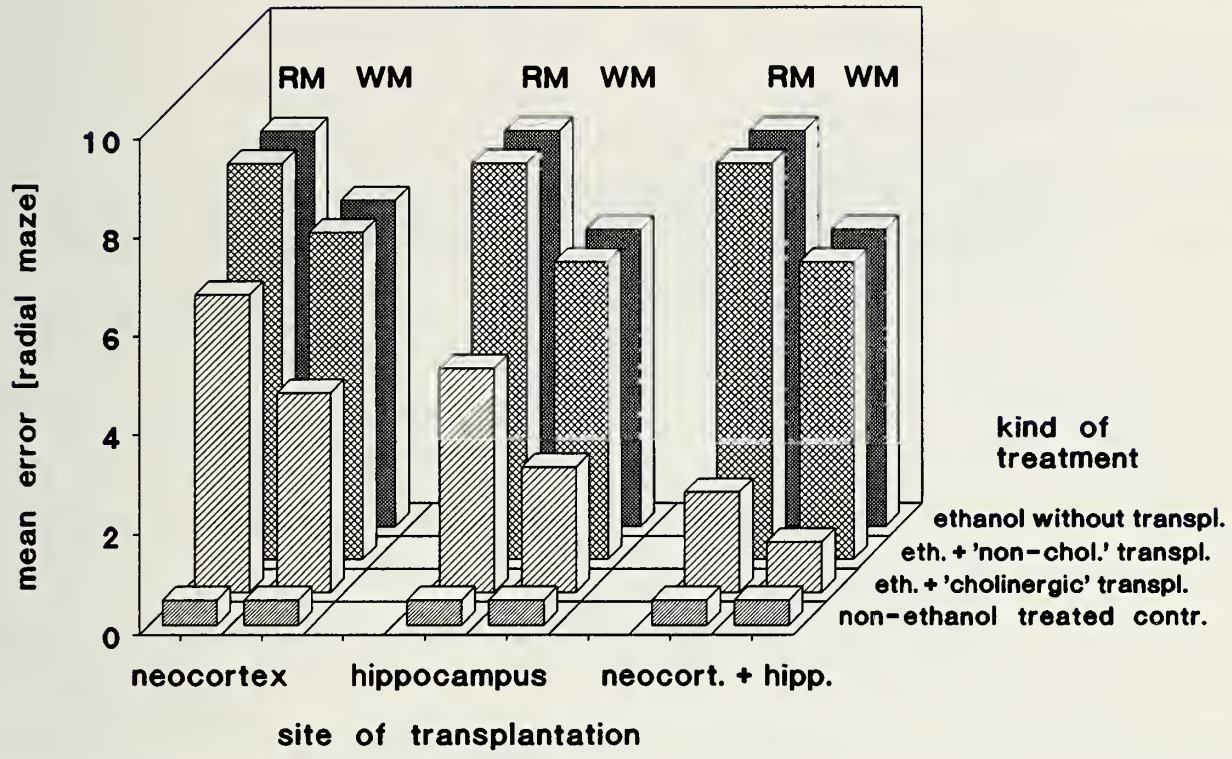

FIGURE 9

Effects of grafting fetal brain tissue on cognitive function of rats previously treated with ethanol (20 percent) for 28 weeks. Transplants were taken either from the hippocampus ("noncholinergic") or from the basal forebrain ("cholinergic") of fetal rat and placed to neocortex or/and hippocampus. Asymptotic performance in the radial arm maze 13 weeks after transplantation. RM: reference memory; WM: working memory. Modified with permission from Arendt et al. (1989*a*).

summarized in figure 11, show that grafting of purified astrocytes is equally effective in improving the performance in the eight-arm radial maze for both reference and working memory as the transplantation of embryonic tissue from the primordial basal forebrain. The rate of behavioral recovery is even more accelerated after grafting of astrocytes. The time course of the improvement in memory function (detected as early as 2 weeks after operation) and the recovery of choline acetyltransferase activity in the basal forebrain (figure 12) makes it most likely that graft-induced behavioral recovery in the present paradigm is due to a neurotrophic influence of astrocytes mediated via the cholinergic basal forebrain system.

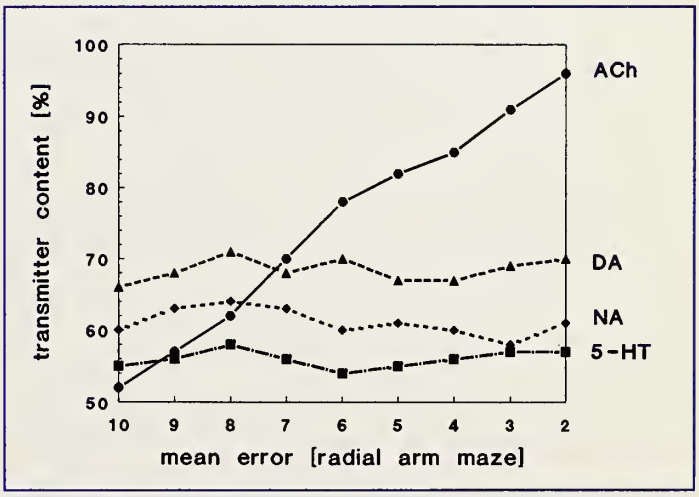

FIGURE 10

Relationship between cognitive performance and changes in transmitter content after grafting of fetal tissue derived from the basal forebrain to the hippocampus of rats previously treated with ethanol (20 percent) for 28 weeks. Each point represents the mean obtained from 5 animals matched for performance in the radial maze at different intervals after operation. ACh, acetylcholine, DA, dopamine; NA, noradrenaline; 5-HT, serotonin.

THE SYNDROME OF PARTIAL CHOLINERGIC DEAFFERENTATION OF THE CORTICAL MANTLE INDUCED BY CHRONIC INTAKE OF ETHANOL

Damage inflicted to the cholinergic neurons of the NbM is parallelled by deficits in a variety of functions, including cortical arousal, selective attention, sleep-wake cycle, and learning and memory (Bartus et al. 1985; Collerton 1986). These behavioral deficits appear to affect most aspects of cognitive function that the cerebral cortex is assumed to subserve. Thus, the physiological mechanisms of the behavioral impairment may not be due to the death of a circumscribed set of basal forebrain neurons per se, but to the malfunction of their cortical target areas. In other words, the behavioral impairment might be related to a functional impairment of the neocortex and hippocampus due to erroneous gaiting or an arousing mechanism normally pro-

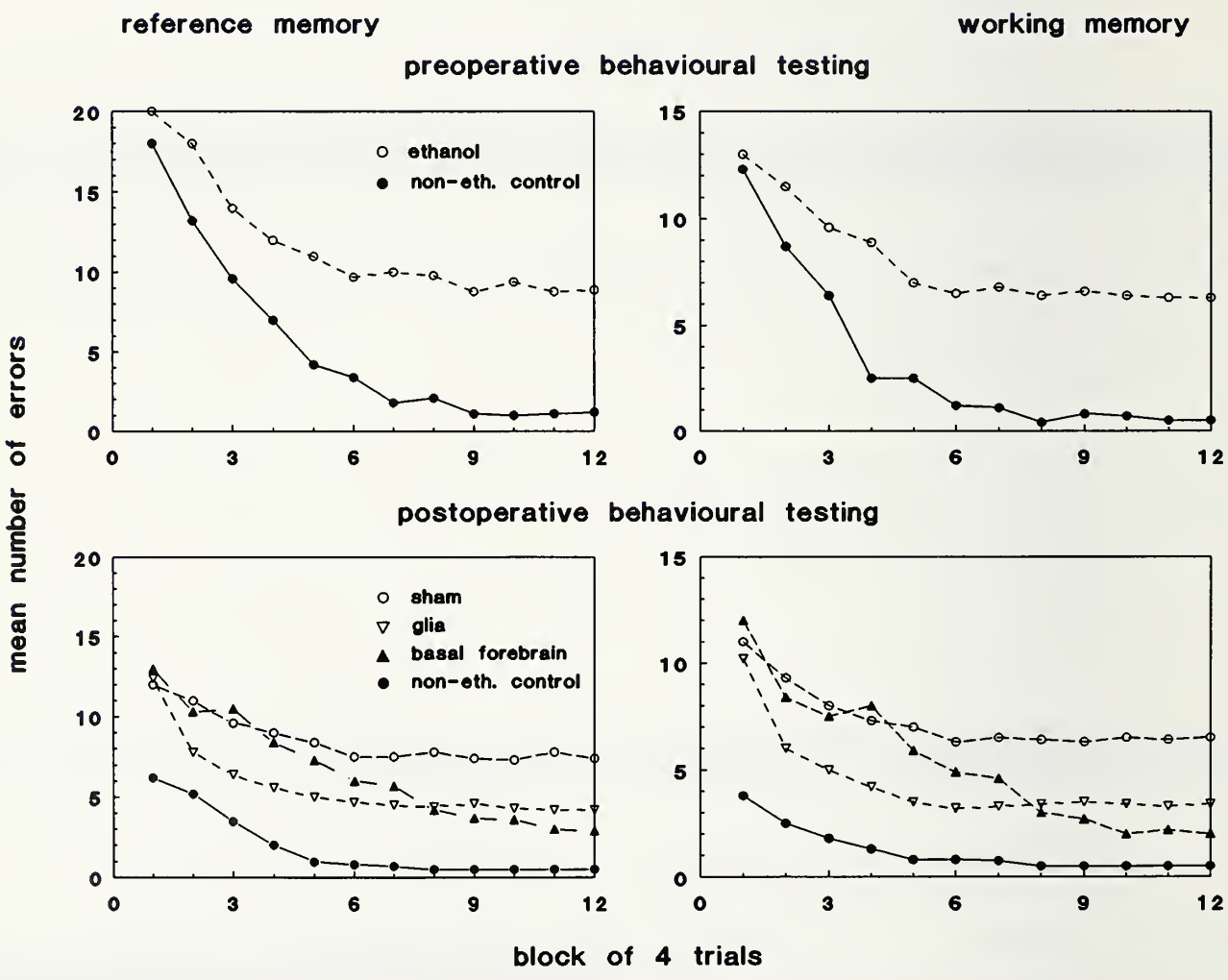

FIGURE 11

Effects of grafts of different cellular origin on the recovery of ethanol-induced memory impairment. Mean number of errors cumulated over blocks of 4 trials in the 8-arm radial maze. Errors made by animals treated with ethanol (20 percent) for 28 weeks were analyzed separately for reference memory and working memory prior to (upper panel) and after (lower panel) receiving grafts of fetal basal forebrain tissue or purified astrocytes simultaneously at 4 sites in the neocortex and hippocampus (group size: n = 8 animals). Modified with permission from Arendt et al. (1989*a*) and Brückner and Arendt (1992).

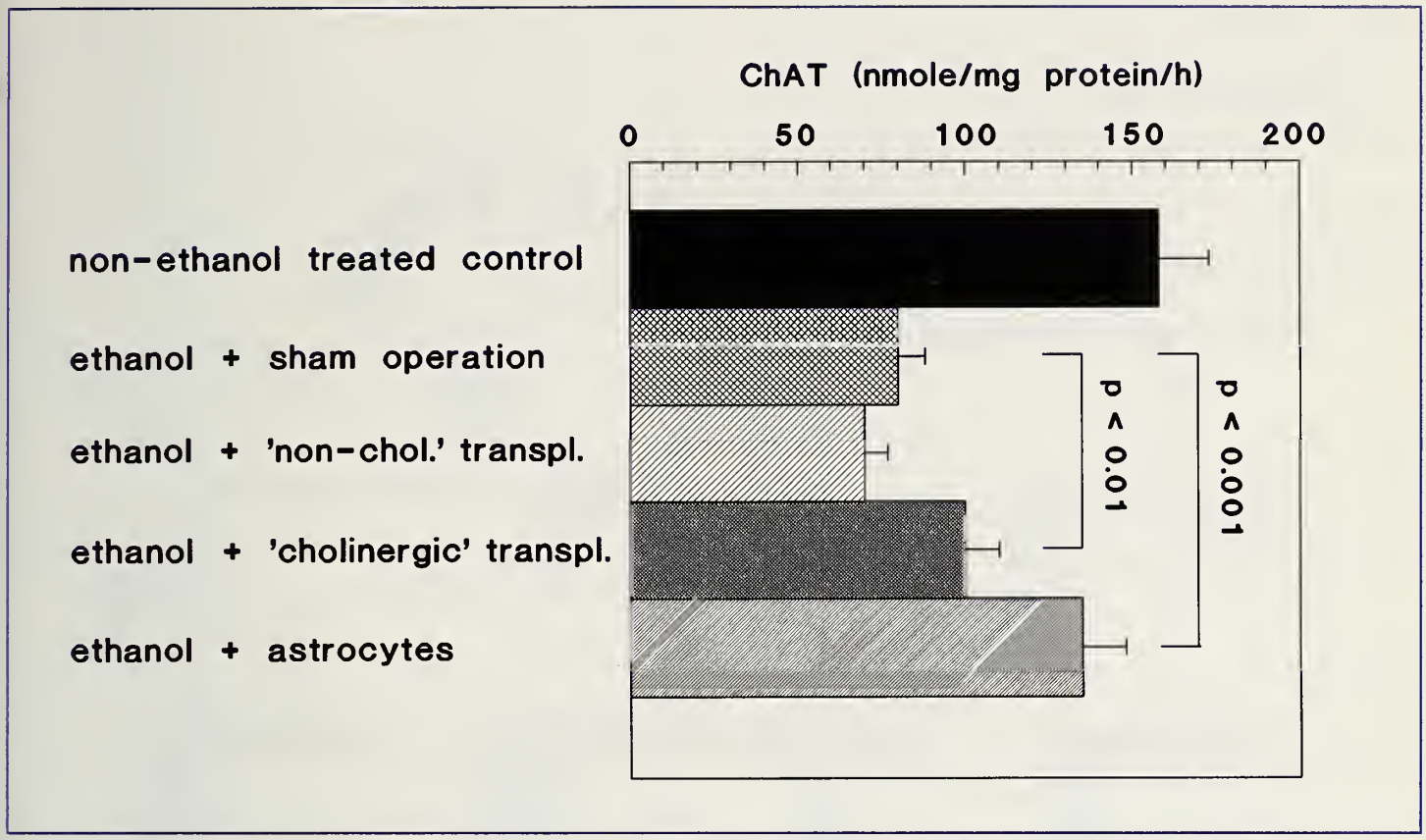

FIGURE 12

Effects of grafts of fetal basal forebrain or of purified astrocytes placed to neocortex and hippocampus on the activity of ChAT in the basal forebrain. Data are mean (± SD), obtained 13 weeks after surgery on the same animals as shown in figure 11 (n = 8). Modified with permission from Brückner and Arendt (1992).

vided by the cholinergic basal forebrain (Steriade and Buzsaki 1990).

Sequelae of a partial cholinergic deafferentation of the cortical mantle concerning impairment in learning and memory can be explained based on the Hippocampal Memory Indexing Theory of Teyler and DiScenna (1986) (figure 13). According to this theory, incoming environmental information is relayed by thalamocortical pathways and engages the activities of numerous cortical modules distributed in space and time over the neocortical surface. The hippocampus subsequently stores this spatiotemporal pattern of active cortical modules by means of long-term potentiation through neocorticolimbic pathways.

According to this theory, only the location and temporal sequencing of activated cortical modules are encoded and not the neuronal transformation of the experiential event itself. The entire information corresponding to the original event, encoded and stored in the hippocampus, is called the hippocampal index. Reactivation of neocortical modules in the appropriate spatiotemporal sequence will stimulate the original experience (recognition memory). If the hippocampal activation exceeds a certain threshold level, the hippocampal index will be activated and, in turn, reactivate the pattern of cortical modules that match the original event (recall memory). As a prerequisite for this processing and

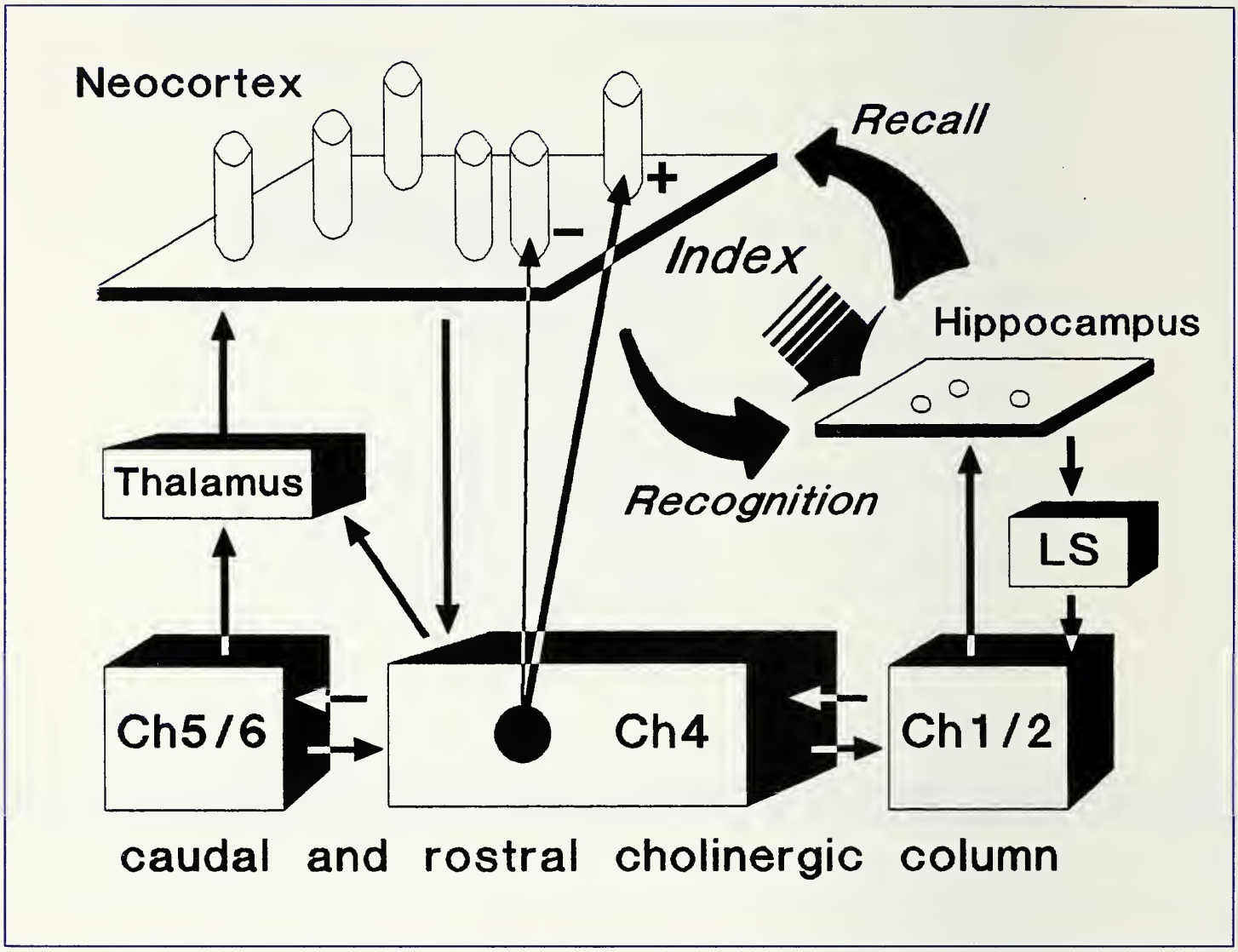

FIGURE 13

Hypothetical involvement of the cholinergic afferentation of the cortical mantle in the processing and storage of information, based on the Hippocampal Memory Indexing Theory of Teyler and DiScenna (1986). Groups of cholinergic projection neurons of the basal forebrain and upper brain stem (Ch nomenclature according to Mesulam 1983*a*,*b*) are reciprocally connected with thalamus, neocortex, and hippocampus as well as with one another and can be viewed as components of the ascending reticular activation system of Moruzzi and Magoun (1949). LS, lateral septum.

encoding of information, a subtle mechanism of tuning thalamic, neocortical, and hippocampal activities, corresponding to the demands of sensory inputs, is needed that is met by the anatomical and electrophysiological properties of the cholinergic projection system of the basal forebrain and upper brain stem.

The complex of symptoms, including cognitive dysfunction, may be quantitatively and qualitatively different depending on the particular site of involvement during the course of the disease (i.e., deafferentation). This complex seen in many dementing disorders (e.g., Alzheimer's disease and progressive supranuclear palsy) and characterized by a common underlying neurobiological substrate can be described as the "syndrome of partial cholinergic deafferentation of the cortical mantle."

As subsystems of the ascending cholinergic system might be involved differently in the pathology of various men-

tal disorders, certain pathoarchitectonic entities can be discriminated. For example, in normal aging, Korsakoff's psychosis, and early Alzheimer's disease, the degeneration is most pronounced for the rostral component, i.e., the septodiagonal band region; whereas progressive supranuclear palsy shows a severe involvement of the caudal component, i.e., the cholinergic system of the upper brain stem (Zweig et al. 1987).

Defining the syndrome of partial cholinergic deafferentation of the cortical mantle as an essential part of mental dysfunction accompanying chronic alcoholism may help in understanding the neurobiological substrates of the major symptomatology. Therefore, this concept may be of heuristic value for developing strategies for the management of this disorder and for understanding the neuronal organization of memory processes under normal and pathological conditions.

DEDICATION

This paper is dedicated to Hartmut Wenk.

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PHARMACOLOGICAL TREATMENT OF ALCOHOL-INDUCED BRAIN DAMAGE

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INTRODUCTION

Significant impairment of brain function is demonstrable in 50 to 85 percent of abstinent alcoholics depending on the clinical setting and the diagnostic technique employed. However, only 10 percent can be diagnosed as having an alcoholism-associated chronic organic mental syndrome (Martin et al. 1986*a*). In addition to cognitive deficits, the chronic alcoholic patient may manifest the symptomatology of mood, anxiety, psychotic, and personality disorders resulting from intoxication/withdrawal, medical or psychiatric complications of alcoholism, or a comorbid psychiatric condition (Berner et al. 1986; Lesch and Nimmerrichter in press). Therefore, the clinician must eliminate the contributions of several factors (e.g., liver disease, aging, head trauma or hypoxemia, psychoactive drug use, depression, or attention deficit disorder) in the diagnostic evaluation of brain impairment in the chronic alcoholic patient. Furthermore, even if these clinical factors have been carefully weighed, and the diagnosis of an alcoholism-associated organic mental disorder is made, one needs to consider nosological distinctions attributable to fundamentally different pathophysiologic mechanisms that may have implications for pharmacotherapy (Martin et al. 1989*b*).

In clinical studies, only indirect methods of measuring the effects of a drug on the human brain can be employed. These methods include psychometric tests, blood or other tissue samples, electroencephalography, and neuroimaging. The term indirect (or surrogate) measure implies some agreement that the measure in question correlates with discrete biological processes of clinical interest. If this relation is carefully established, a thorough evaluation of the effects of a drug in well-characterized patients might

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elucidate pathophysiologic processes in the human brain.

The following review evaluates 15 years of clinical studies in which cognitive enhancers were investigated as treatment for alcohol-induced cognitive dysfunction. To explain why the results of these studies vary so widely, particular attention is paid to methodological factors and the characteristics of the patient populations. Limited reference is made to animal experiments when they enhance our understanding of the pathophysiology or pharmacotherapy of alcoholism-associated brain damage. The basic tenet of this paper is that carefully interpreted findings from clinical trials can provide certain insights into alcohol-related cognitive dysfunction and its treatment. These findings, in turn, can generate testable hypotheses concerning etiopathogenesis. This iterative process may eventually lead to a better understanding of the specific pathophysiologic mechanisms underlying alcoholic organic mental disorders and may have broad application to other neuropsychiatric diseases.

STUDIES OF THE PHARMACOTHERAPY OF ALCOHOLIC ORGANIC MENTAL DISORDERS

In table 1, studies are listed in which psychopharmacologic agents were administered as cognitive enhancers to chronic alcoholics with cerebral impairment. Daily dosage and duration of drug administration, diagnoses and numbers of patients studied, diagnostic criteria, reported treatment outcome, and outcome measures are indicated for each study.

Pharmacologic Treatments

Drug trials of cognitive dysfunction in the chronic alcoholic have most frequently been based on putative neurotransmitter abnormalities. However, the usefulness of less specific drugs that improve cerebral circulation and blood oxygenation and/or cause metabolic activation has also been explored (Martin et al. 1989*b*). Treatment studies to date have shown few consistent findings and suggest that, besides pharmacologic factors, diagnostic and methodologic issues need consideration to interpret the results.

Trial Design

All but two of the reviewed studies were double-blind and placebo controlled. Several studies employed a crossover design (Franceschi et al. 1982; Jenkins et al. 1982; Langlais et al. 1988; LeBoeuf et al. 1978; Mair and McEntee 1986; Martin et al. 1984*a*,*b*, 1989*a*; McEntee and Mair 1980; O'Donnell et al. 1986), adding statistical power to the often small (1 to 8 patients) sample sizes. The larger (14 to 60 patients) studies (Barnas et al. 1987, 1990; Laczi et al. 1983; Saletu et al. 1978, 1983, 1990) employed the preferred parallel design. However, direct comparison of these smaller and larger studies may be impossible because the patient populations likely are quite different.

Diagnostic Issues

The diagnostic criteria used in the studies cover a broad range of classification

TABLE 1

Clinical trials of cognitive enhancers administered to chronic alcoholics with organic brain impairment

Author	Drug (dose/day)	Duration of drug administration (weeks)	Diagnosis (n)[1]	Diagnostic criteria[2]	Improvement	Reported outcome measures[3]
Saletu et al. 1978	EMD 21657 (900 mg)	6	D/W (40)	Clinical (VRC)	+	Clinical, memory, psychopathology, EEG
LeBoeuf et al. 1978	Vasopressin (22.5 IU)	2	KS (1)	Psychometric (WAIS-WMS > 20)	+	Clinical, memory
Blake et al. 1978[4]	Vasopressin (16 IU)	2, 3	KS (2)	Clinical	–	Clinical, memory
Oliveros et al. 1978[4]	Vasopressin (11 IU)	3	KS (1)	Clinical	+	Clinical, psychopathology
McEntee and Mair 1980	Clonidine (0.6 mg) D-Amphetamine (20 mg) Methysergide (8 mg)	2 2 2	KS (8)	Clinical (Victor et al. 1989) Psychometric (WAIS-WMS ≥ 20)	+ – –	Memory
Franceschi et al. 1982	Vasopressin (15 IU) Physostigmine (1 mg)	1 Single dose	KS (6)	Psychometric (WMS 50-75)	– –	Memory, psychopathology
Jenkins et al. 1982	Desmopressin (160 µg)	1	KS (1)	Clinical	–	Memory
Laczi et al. 1983	Desglycinamide-arginine-vasopressin (80 µg)	1	KS (14)	Clinical (Victor et al. 1989, Squire 1982)	–	Memory, psychopathology blood chemistry
Saletu et al. 1983	Piridoxilate (600 mg)	6	D/W (36)	Clinical (VRC)	+	Clinical, memory, psychopathology, EEG, piridoxal-5-phosphate level
Martin et al. 1984*a*, 1984*b*	Clonidine (6–12 µg/kg)	3	KS (7)	Clinical (Victor et al. 1989, DSM-III) Psychometric	–	Clinical, memory, psychopathology, biogenic amines, blood pressure, pulse

TABLE 1 *CONT'D*

Clinical trials of cognitive enhancers administered to chronic alcoholics with organic brain impairment

Author	Drug (dose/day)	Duration of drug administration (weeks)	Diagnosis (n)[1]	Diagnostic criteria[2]	Improvement	Reported outcome measures[3]
Mair and McEntee 1986	Clonidine (0.8 mg) L-DOPA (400 mg) Ephedrine (100 mg)	2 2 2	KS (7)	Clinical (Victor et al. 1989) Psychometric (WAIS-WMS μ 20)	+ – –	Memory, psychopathology, biogenic amines
O'Donnell et al. 1986	Methylphenidate (0.2 mg/kg) Physostigmine (1 mg) Methylphenidate (20 mg) Choline chloride (10.4–18.2 g) Methylphenidate+ Choline chloride	Single dose Single dose 1 1 1	KS (6)	Clinical (DSM-III)	– – + – –	Memory
Barnas et al. 1987	Piracetam (6 vs. 24 g)	6	D/W (39)	Clinical Psychometric	–	Memory, psychopathology,
Langlais et al. 1988	DL-threo-3, 4-dihydroxyphenylserine (1 g)	Single dose	KS (8)	Clinical (Victor et al. 1989) Psychometric (WAIS-WMS ≥ 20)	–	Memory, blood pressure, pulse
Martin et al. 1989*a*	Fluvoxamine (100–200 mg)	4	KS (6) DAA (3) LD (1)	Clinical (DSM-IIIR) Psychometric	+	Memory, biogenic amines, fluvoxamine level
Barnas et al. 1990	Piracetam (6 vs. 24 g)	6	D/W (60)	Clinical (DSM-III-R) Psychometric	+	Memory, psychopathology
Saletu et al. 1990	Modafinil (200 mg)	6	D/W (38)	Clinical (VRC)	+	Clinical, memory, psychopathology, EEG

Notes:
[1]*Diagnoses include detoxification/withdrawal (D/W); Korsakoff's syndrome (KS); Dementia associated with alcoholism (DAA); compensated liver disease (LD).*
[2]*Diagnostic criteria abbreviations are Vienna Research Criteria (VRC); Wechsler Adult Intelligence Scale (WAIS); Wechsler Memory Scale (WMS); Diagnostic and Statistical Manual, Third Edition, 1980 (DSM-III); Diagnostic and Statistical Manual, Third Edition, Revised, 1987 (DSM-III-R)*
[3]*Psychopathology includes all psychic functions termed by authors as vigilance, attention, concentration, attention variability, drive, mood, and affect that can modulate memory.*
[4]*Design not double blind.*

systems. This diversity reflects a lack of consensus in terminology due to the diverse clinical orientations of researchers in the field, differing diagnostic traditions in the countries in which the studies were conducted, newer nosologic concepts related to technologic developments, and perhaps even changes in the clinical presentation of these disorders from classical descriptions (Harper and Kril 1990; Lishman 1981; Martin et al. 1986*a*).

For the most part, three different groups of patients comprise the study populations in the reviewed clinical trials. The first rather heterogeneous group includes alcoholics abstinent for less than 3 weeks (usually 1 week) when the therapeutic trials started. This group is the detoxification/withdrawal (D/W) group and includes patients diagnosed according to the Viennese Research Criteria (Berner 1977) as having organic brain syndrome (Saletu et al. 1978, 1983, 1990), diagnosed according to the DSM-III-R criteria (American Psychiatric Association 1987) as having substance-induced organic mental disorder (Barnas et al. 1990), or diagnosed and verified by psychometric testing as having simply "cognitive impairment" (Barnas et al. 1987). Although D/W patients may manifest a syndrome of organic cerebral impairment, including reduced intellectual functioning and memory deficits with significant affective symptoms within the first week of abstinence, only approximately 10 percent of alcoholics will remain grossly impaired after 3 to 6 weeks of abstinence (Eckardt and Martin 1986; Feselmayer et al. 1983; Lesch 1985). This conclusion is supported by the spontaneous improvement observed in the reviewed studies, regardless of whether D/W patients received active medication or placebo (Barnas et al. 1987, 1990; Saletu et al. 1978, 1983, 1990).

Brain impairment in the chronic alcoholic after 3 weeks of abstinence has been heuristically conceptualized (American Psychiatric Association 1987; Lishman 1981; Martin et al. 1986*a*) as two clinically distinguishable organic mental disorders: alcohol amnestic disorder and dementia associated with alcoholism (DAA). Alcohol amnestic disorder, commonly called Korsakoff's syndrome (KS), is characterized by an amnestic syndrome in which short-term and long-term memory impairments and behavioral changes occur without clouding of consciousness or general loss of major intellectual abilities. DAA consists of global loss of intellectual abilities, with an impairment in memory function and disturbance(s) of abstract thinking, judgment, other higher cortical functions, or personality changes without a clouding of consciousness. If patients were abstinent for at least 3 weeks at the time of study, we accepted the authors' diagnosis of either KS or DAA. However, in only a few studies (Barnas et al. 1990; Langlais et al. 1988; Mair and McEntee 1986; Martin et al. 1984*a*,*b*, 1989*a*; McEntee and Mair 1980) did the authors report using strict clinical and psychometric criteria for these diagnoses. In addition, only a few DAA patients have ever been studied in pharmacotherapeutic trials (Martin et al. 1989*a*).

Outcome Measures

Measures of memory functioning were used to judge the efficacy of the psychopharmacologic interventions in alcoholic organic mental disorders in all but one (Oliveros et al. 1978) of the reviewed studies. Factors that modulate and influence cognitive performance (denoted in table 1 as "psychopathology"), such as vigilance, concentration, attention, arousal, motivation, and drive, as well as affect and mood, were only used in half the studies (Barnas et al. 1987, 1990; Franceschi et al. 1982; Laczi et al. 1983; Mair and McEntee 1986; Martin et al. 1984*a*,*b*, 1989*a*; Oliveros et al. 1978; Saletu et al. 1978, 1983, 1990). Several objective measures of drug effects are available. These include the pharmacoelectroencephalogram (EEG) (Saletu et al. 1978, 1983, 1990); concentrations of biogenic amines or peptides in cerebrospinal fluid (CSF), blood, or urine (Martin et al. 1984*a*,*b*, 1989*a*; Mair and McEntee 1986); and hemodynamic (pulse or blood pressure) effects (Langlais et al. 1988; Martin et al. 1984*a*,*b*). However, these measures are seldom reported. Furthermore, blood levels of the drugs under investigation, which are useful in interpreting pharmacokinetic aspects of interindividual differences in treatment response, are reported in only two studies (Martin et al. 1989*a*; Saletu et al. 1983). Finally, although clinical descriptions are frequently mentioned, validated scales documenting clinical benefit have only been employed in a few studies (Saletu et al. 1978, 1983, 1990; Martin et al. 1984*b*).

TREATMENT EFFICACY RELATED TO PHARMACOLOGIC CLASSIFICATION OF COGNITIVE ENHANCERS

The results reviewed in table 1 of the clinical trials using cognitive enhancers to treat alcohol-induced brain damage are grouped in table 2 according to the putative major mechanism of pharmacologic action of the drug. The number of studies in which each drug was administered, the total number of patients receiving the medication, and their diagnoses are also shown in the table. Although these studies are not strictly comparable because of methodologic reasons (see above), this type of summation may suggest hypotheses concerning etiopathogenesis. Of course, drugs may alter behavior through a variety of mechanisms (both direct and indirect), and not all patient groups may respond equivalently to a given drug.

AGENTS THAT MODIFY NEUROTRANSMITTER ABNORMALITIES

Noradrenergic Drugs

In both animals and humans, noradrenergic drugs may facilitate memory processes, at least partly, by affecting such modulatory processes as arousal or attention (Mair and McEntee 1986; Petersen 1992). These effects could result from increasing the firing rate of certain neurons in the nucleus coeruleus (Olpe et al. 1985). Although McEntee and Mair (1978) reported a significant correlation between the memory impairment of chronic KS patients and

TABLE 2

Outcomes of clinical trials of drug classes of cognitive enhancers in alcohol-induced brain damage

Drug class	Studies[1] (n)	Diagnosis[2] (n)	Outcome[3]
Neurotransmitter abnormalities			
Noradrenergic			
Clonidine	3	KS (22)	+,–
DOPS[4]	1	KS (8)	–
D-Amphetamine	1	KS (8)	–
Ephedrine	1	KS (7)	–
Modafinil	1	D/W (20)	+
Dopaminergic			
Methylphenidate	1	KS (6)	+
L-DOPA	1	KS (7)	–
Serotonergic			
Fluvoxamine	1	KS (6)	+
		DAA (3)	–
		LD (1)	–
Methysergide	1	KS (8)	–
Cholinergic			
Physostigmine	2	KS (12)	–
Choline chloride	1	KS (6)	–
Peptides			
Vasopressin	4	KS (10)	+,–
DGAVP[5]	1	KS (8)	–
Desmopressin	1	KS (1)	–
Cerebral metabolism			
Piracetam	2	D/W (40)	+,–
Piridoxilate	1	D/W (18)	+
EMD 21657	1	D/W (19)	+

Notes:

[1] *Number of studies conducted using a specific drug.*

[2] *Diagnoses as in table 1. Only those patients who received the active drug (not the placebo) are included.*

[3] *Beneficial (+) and no beneficial (–) effects, as judged by authors.*

[4] *DL-threo-3,4-dihydroxyphenylserine.*

[5] *Desglycinamide-arginine-vasopressin.*

reduced CSF concentration of the major metabolite of norepinephrine, 3-methoxy-4-hydroxyphenylglycol (MHPG), these findings have not been replicated by other investigators (Martin et al. 1984*c*, 1989*a*; Wood et al. 1982).

McEntee and Mair (1980) found that the α_2-adrenergic agonist clonidine produced a statistically significant improvement in memory in a 2-week, placebo-controlled, double-blind, counterbalanced drug trial in eight patients with KS. Treatment with the serotonin antagonist methysergide or the sympathomimetic d-amphetamine resulted in no significant changes across subjects compared to treatment with placebo. However, there were considerable interindividual differences in response among patients.

In a second study (Mair and McEntee 1986), only clonidine (but not levodopa or ephedrine) significantly improved anterograde amnesia. Each medication affected some measures of attention, but none of the treatments had an effect on retrograde amnesia or digit-symbol substitution. Patients with the lowest CSF concentrations of MHPG tended to have the greatest improvement during clonidine treatment.

In contrast to these findings, clonidine therapy, at drug doses with predicted effects on hemodynamic parameters and central nervous system (CNS) biogenic amine metabolism, had no significant effect on either semantic or episodic memory in seven patients with KS using a placebo-controlled, double-blind, double-crossover design (Martin et al. 1984*a*,*b*,*c*). However, episodic memory was significantly improved during clonidine withdrawal compared to both drug and baseline conditions without evident changes in mood or new psychological symptoms, which are important considerations due to the similarities in episodic memory impairment in depression and KS (Weingartner et al. 1983*a*). On the other hand, access to semantic memory was unaffected by discontinuation of clonidine treatment.

Although chronic treatment of Parkinson patients with the nonphysiologic norepinephrine precursor DL-threo-3,4-dihydroxyphenyl-serine (DOPS) elevated mood and diffusely activated mental functions (Narabayashi et al. 1981), Langlais et al. (1988) found no significant effects on memory when DOPS was administered as a single dose in eight long-term amnesic KS patients.

In D/W alcoholics (7 to 10 days of abstinence) treated with the central α_1-adrenergic agonist modafinil, spontaneous recovery of psychopathology related to organic brain syndrome, other than memory functions, progressed more rapidly than in the placebo group (Saletu et al. 1990). These beneficial effects resulted largely from improved vigilance, as suggested by the increase on electroencephalogram (EEG) in relative power of alpha activity and decrease in delta and theta after a modafinil dose during the sixth week of treatment.

Dopaminergic Drugs

Although destruction of the dopaminergic nigrostriatal pathway does not, in itself,

disrupt retention (Fibiger and Phillips 1976), electrical stimulation of this pathway can alter dopaminergic influences on cholinergic neurons (Squire and Davis 1981). Dopaminergic pathways, especially in the nigrostriatal system, may be involved in motor learning and in mediating the effects of reward (Squire and Davis 1981).

O'Donnell et al. (1986) compared the memory effects of methylphenidate, which enhances release and blocks reuptake of dopamine and norepinephrine in mammalian brain, to those of the cholinergic drugs choline chloride and physostigmine in six KS patients. Long-term memory improved significantly after 3 weeks of methylphenidate treatment. However, memory was not improved after single doses of these drugs or 3 weeks' administration of choline chloride.

Serotonergic Drugs

Research on aging and Alzheimer's disease has emphasized the role of the serotonergic system in memory processes (McEntee and Crook 1991). Studies in thiamine-deficient animals (Martin et al. in press) as well as those of sleep (Martin et al. 1986*b*), neuroendocrine (Eisenhofer et al. 1984; Branchey et al. 1985), and memory (Weingartner et al. 1983*b*) functions in alcoholics with organic mental disorders suggested the potential therapeutic value of enhancing serotonergic neurotransmission in this patient population. The serotonin uptake blocker fluvoxamine was administered to 10 patients in a 4-week, double-blind, crossover design (Martin et al. 1989*a*). This drug was chosen because of its potency and high selectivity, and because it does not possess metabolites that act at the norepinephrine uptake site. All patients displayed severe deficits in memory for recently acquired information (episodic memory). Only the DAA patients showed global intellectual decline including decreased performance on measures of semantic (knowledge) memory. Fluvoxamine treatment improved episodic memory *only* in the KS patients. This treatment in these patients also produced the predicted fall in CSF concentrations of the major serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA), which correlated significantly with blood fluvoxamine levels.

A significant correlation was also found between fluvoxamine-associated improvement on memory tests and decreases in CSF 5-HIAA. For DAA, even with adequate blood levels of fluvoxamine, no changes in CSF 5-HIAA and memory function were observed. These findings suggest that with individual titration of the drug dose, fluvoxamine might be a clinically useful agent in the treatment of KS.

Cholinergic Drugs

The "cholinergic hypothesis" has been the basis for research into the treatment of Alzheimer's disease (Mayeux 1990). Strategies employed for augmenting central cholinergic neurotransmission include using precursors (e.g., choline, lecithin, or acetyl-L-carnitine), blocking cholinesterase (e.g., physostigmine or tetrahydroaminoacridine), increasing the presynaptic release of acetylcholine (e.g.,

piracetam and related nootropic agents), and trying various combinations of presynaptic and postsynaptic drugs. Because of parallels between Alzheimer's disease and DAA (Martin et al. 1989*b*) and the effects of thiamine deficiency (Barclay et al. 1981) and chronic alcohol administration (Arendt et al. 1988) on central cholinergic function, these strategies may also be effective in treating chronic organic mental disorders associated with alcoholism (see Arendt, chapter 22). However, cholinergic substances have not been studied in DAA patients, and in the studies reviewed here have no beneficial effects in KS (Franceschi et al. 1982; O'Donnell et al. 1986).

Peptides

Peptides and peptide fragments lacking classical endocrine activity can influence some forms of adaptive behavior via CNS mechanisms (De Wied and Gispen 1977). Several peptides are thought to influence learning and memory, with vasopressin and its analogues having received most attention. Evidence suggests that vasopressin affects memory via noradrenergic pathways in limbic-midbrain structures (van Wimersma and De Wied 1976). However, since it is unknown whether vasopressin and its analogues cross the blood-brain barrier after systemic or nasal administration (Jenkins et al. 1982), a role in humans for these peptides in learning and memory has not been clearly established. Initial research produced largely mixed results (LeBoeuf et al. 1978; Oliveros et al. 1978; Blake et al. 1978). However, later studies have reported mostly negative findings (Franceschi et al. 1982; Jenkins et al. 1982; Laczi et al. 1983). Furthermore, cognitive benefits of vasopressin have been attributed to improvements in mood, attention, and arousal (Kopelman and Lishman 1986).

AGENTS THAT INFLUENCE CEREBRAL METABOLISM

Piracetam, a cyclic derivative of the neurotransmitter γ-aminobutyric acid (GABA) that does not possess GABA-like properties, may provide beneficial effects in various causes of organic cerebral impairment (including aging, Alzheimer's disease, early parkinsonism, acute cerebral ischemia, and postconcussional syndrome) by stimulating cholinergic mechanisms as well as by nonspecific activation of cerebral metabolism (Froestl and Maitre 1989; Pepeu et al. 1989). Barnas et al. (1987) initially investigated the effects of piracetam on attention, concentration, critical flicker frequency, short-term memory, and intelligence in 39 D/W (abstinent for 7 to 10 days) alcoholic patients with organic mental disorder, verified by neuropsychological testing. Concentration and attention spontaneously improved over time in all patients, but no beneficial effects were observed for piracetam at either dose compared to a placebo.

In a second report (Barnas et al. 1990), statistical analysis of the psychometric findings from 60 D/W alcoholics (expanded in terms of "speed" and "power" factors) showed that speed-related psychometric variables which are highly influenced by training improved over

time in all three groups. However, a higher dose of piracetam significantly improved the power factor.

Saletu et al. (1978, 1983) conducted two large, 6-week, double-blind, placebo-controlled studies, using clinical, psychometric, and computer-assisted EEG spectral analysis. They demonstrated accelerated and augmented spontaneous recovery of cerebral dysfunction in abstinent D/W alcoholic patients by administering antihypoxidotic nootropic drugs. The clinical benefits from these nootropic agents may relate to their vigilance-improving properties derived from more efficient brain bioenergetics.

INSIGHTS CONCERNING PATHOGENESIS AND NOSOLOGY

Classification of patients with alcoholic organic mental disorders has been based on clinical characteristics such as history, neuropsychological test performance, and longitudinal course (Martin et al. 1986*a*). Memory impairment (amnesia) is nosologically significant in most approaches to classification, including the DSM-III-R diagnostic criteria of the American Psychiatric Association (1987). It is equally important to determine whether a patient has developed other intellectual deficits or psychopathology as a consequence of chronic alcoholism.

It has been suggested that subcortical periventricular lesions due to alcoholism-related thiamine deficiency are pathognomonic of alcohol amnestic disorder. On the other hand, dementia associated with alcoholism is associated with cortical atrophic changes resulting from ethanol neurotoxicity (Lishman 1981; Martin et al. 1986*a*). However, these two organic mental disorders are not mutually exclusive, and some features of each often coexist in the same patient. Whether it is nosologically meaningful to differentiate these clinical syndromes of impairment remains controversial (Bowden 1990; Victor et al. 1989) and awaits findings from clinical trials in which patients are carefully characterized using research methods for assessing altered CNS structure and function (Martin et al. 1989*b*).

Differential Diagnosis

Every alcoholic patient should have a complete neuropsychiatric evaluation. This should be done to determine the relative contributions of various pathological processes underlying organic cerebral impairment and to eliminate disorders requiring specific therapeutic measures (Cummings and Benson 1992). Alzheimer's disease, multi-infarct dementia, depression or pseudodementia, traumatic head injury, and chronic liver dysfunction are the most common other diagnostic entities that must be considered. The initial diagnostic task is to learn which psychopathological features persist once the signs of intoxication and withdrawal have abated. Due to the neurophysiologic disequilibrium of the alcohol withdrawal syndrome, waiting at least 3 weeks seems prudent to establish reliable baseline measures of neuropsychological impairment and psychopathology (Eckardt and Martin 1986). The situation is complicated further by the fact that elderly patients may require prolonged

periods of detoxification, compared with younger individuals, to stabilize brain functioning (Goldman 1980).

Neuropsychological Testing

The neuropsychological examination, which complements clinical and laboratory evaluations, can provide a specific diagnosis and evaluate the severity of the cognitive deficits (Eckardt and Martin 1986). Tests of various neurobehavioral functions can help differentiate between pathophysiologic processes that preferentially affect the cerebral cortex (e.g., Alzheimer's disease, DAA) or subcortical diencephalic and brain stem systems (e.g., Parkinson's disease, depression, KS) (Cummings and Benson 1992). Psychometric criteria that distinguish between memory and global intellectual functioning are commonly used to operationalize the diagnosis of KS. However, since both KS and DAA patients have memory dysfunction, the diagnostic focus preferably should be determining characteristics of the memory deficits (Weingartner et al. 1983*a*).

Electrophysiologic Studies

The clinical EEG is often abnormal in dementia, with diffuse symmetrical slowing of dominant frequencies and a correlation between the amount of slow-wave activity and degree of cognitive deficits. Contrary to this finding, many KS patients (even those with severe amnesia) have normal EEGs. Evoked potential studies may be particularly useful for differentiating cortical and subcortical pathophysiologic processes present in DAA and KS, respectively (Goodin and Aminoff 1986).

Brain Imaging

Cerebellar degeneration, symmetric midline hemorrhagic, and/or necrotic lesions located near the third and fourth ventricles and aqueduct of Sylvius, and reduction in brain tissue, particularly in the superior frontal cortex, have been observed in autopsy studies of chronic alcoholic patients (Harper and Kril 1990; Victor et al. 1989; see also Harper and Kril, chapter 3). Diagnostically useful brain magnetic resonance imaging (MRI) techniques are now available to identify localized atrophy of the mammillary bodies (a relatively specific finding in KS) and hippocampal regions (frequently damaged in cortical dementias, including Alzheimer's disease) (Squire et al. 1990). Regional cerebral blood flow (CBF), positron emission tomography (PET), single photon emission computerized tomography (SPECT), and topographic brain mapping by EEG can measure various aspects of brain functioning. Therefore, they are likely to find their greatest utility in elucidating the mechanisms and evaluating the outcomes of treatments (see Pfefferbaum and Rosenbloom, chapter 4).

Biological Markers

Increased understanding of the molecular pathophysiology of alcoholic organic brain disease may allow the development of laboratory methods to identify individuals at genetic risk (Martin and Charness in press). Furthermore, measurement of changes in various blood or CSF con-

stituents (neurotransmitter, peptide, or protein concentrations) may allow quantitation of the severity of brain damage as well as the response to treatment. However, the specificity of such changes to alcoholism-associated mental disorders should be shown before use in differential diagnosis.

PATIENT HETEROGENEITY AND TREATMENT APPROACHES

The clinical and psychobiologic determinants guiding pharmacologic treatment of KS and DAA suggest considerable overlap between these disorders and some shared features with other neuropsychiatric diseases (Martin et al. 1989*b*). However, the heterogeneity of the response to pharmacotherapy of cognitively impaired chronic alcoholics supports the need to identify patient groups in whom alternate modes of treatment are indicated based on fundamental differences between groups.

Trial Design and Outcome Measures

The magnitude and character of spontaneous recovery of brain function and large interindividual differences among patients (Eckardt and Martin 1986; Unkenstein and Bowden 1991) must be considered when one judges the efficacy of any treatment modality for alcoholic organic brain disease. Hence, patients must be studied after prolonged abstinence or using a parallel design in which one group receives a placebo. Consideration of several variables, such as the degree of abstinence, psychosocial support, concomitant medical illnesses, and psychological components of the treatment, may require long-term studies with large sample sizes. Tests of neurobehavioral functioning must be carefully selected to measure both amnestic and dementia components of cognitive dysfunction and to allow differentiation of the effects of practice from spontaneous recovery. By definition, these neuropsychological tests provide the major outcome measures to which electrophysiological, brain imaging, and biochemical markers must be correlated.

Current Pharmacologic Treatments

The cornerstone of treatment should be abstinence and proper nutrition. Any pharmacologic approaches should, above all, not retard normal CNS recovery that occurs with abstinence (Martin et al. 1986*a*). Behavioral retraining methods will have an important place in management of patients with chronic organic mental disorders associated with alcoholism. Drug trials in the chronic alcoholic with cerebral impairment have shown inconsistent results among studies and considerable interindividual differences among patients in response to treatment. Improvements in memory functioning have also been demonstrated in some (but not all) short-term studies with both catecholaminergic and serotonergic agents (Martin et al. 1989*a*; McEntee and Mair 1990). However, it may be unreasonable to assume that a single pharmacologic approach will be adequate in patients as diverse as those with chronic organic mental disorders associated with alcoholism. The heuristic value of considering whether global dementia or amnesia is the preponderant clinical finding in the

chronic alcoholic is underlined by the differential effects of fluvoxamine in patients with KS and DAA (Martin et al. 1989*a*).

FUTURE DIRECTIONS

Long-term studies have not been conducted to determine whether *any* pharmacologic intervention alters the natural outcome of chronic organic mental disorders associated with alcoholism. There is a dearth of clinical trials with DAA patients. Nootropic drugs that improve cerebral bioenergetics have not been evaluated in patients with stable cognitive deficits. Since small benefits may be anticipated in patients in whom brain functioning is severely compromised, preventive strategies to *reduce* CNS damage based on similarities between neurodegenerative disorders and alcoholic organic brain disease (Arendt et al. 1988; Langlais and Mair 1990; Vogel and Hakim 1988) may become increasingly important. Because of the demonstrated continuum of cognitive impairments and certain shared neuropathologic abnormalities, there is great potential in exploring in less impaired alcoholics the effects of pharmacological approaches showing some promise in alcoholic patients with severe cognitive deficits (Martin et al. 1986*a*). Particularly desirable would be drugs that modify the usual clinical course of alcoholism, characterized by repeated episodes of ethanol use, malnutrition, and withdrawal and progression of organic cerebral impairment.

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